PL1 primer PL5' primer

acaaagcccacttgtattcgcaagâ◆ATGAAGTTCACTGCCACCTTGCTCATGATGGCCATCTTCGTCCTC

M K F T A T F L M M A I F V L

↓

ATGGTTGAACCTGGAGAGTGTGGCTGGGGAAGCTTTTTTAAAAAGGCTGCTCACGTTGGCAAGCATGTTGGC

M V E P G E C G W G S F F K K A A H V G K H V

G

PL3' primer

↓

◆

AAGGCGGCCCTTACTCATTACCTTGGCGATAAGCAGGAGCTCAACAAGCGTGCAGTCGATGAAGACCCAAAT

K A A L T H Y L G D K Q E L N K R A V D E D P

N

PL2 primer

GTTATTGTTTTTGAATGAagaaatcgccttgaaggagccttcagatgatataataatccttcttgctttaat

V I V F E *

gaaataaatcagactttacctgcaacagcaaaaaaaaaaaaaaaa

Fig. 1a

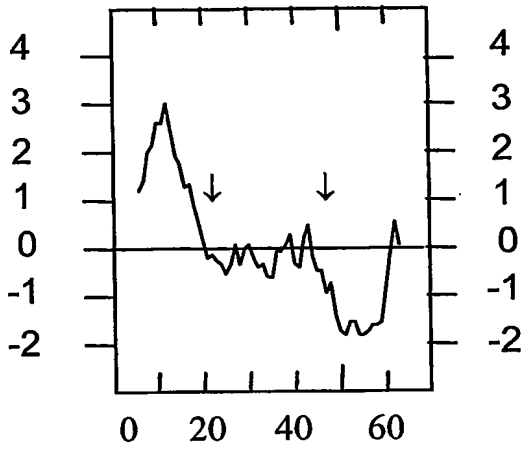
4
3
2
1
0
-1
-2
0 20 40 60
Amino acid residue

Fig. 1b

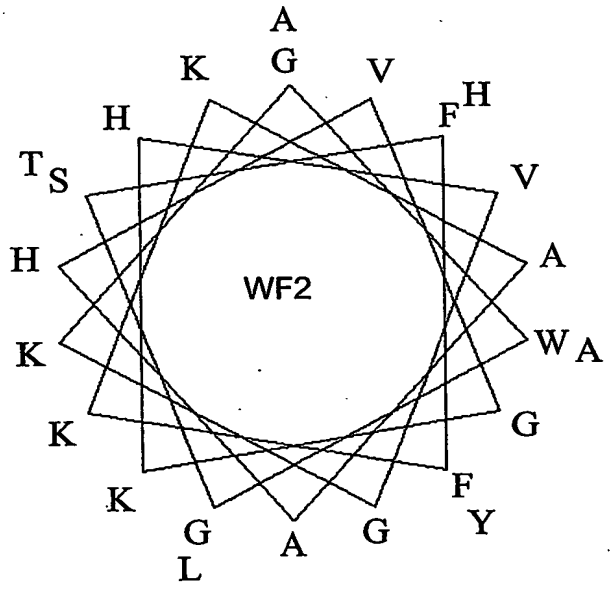
A
G
K
V
H
F
H
T
S
V
H
A
WF2
K
W
A
K
G
K
F
Y
G
L
A
G

Fig. 1c

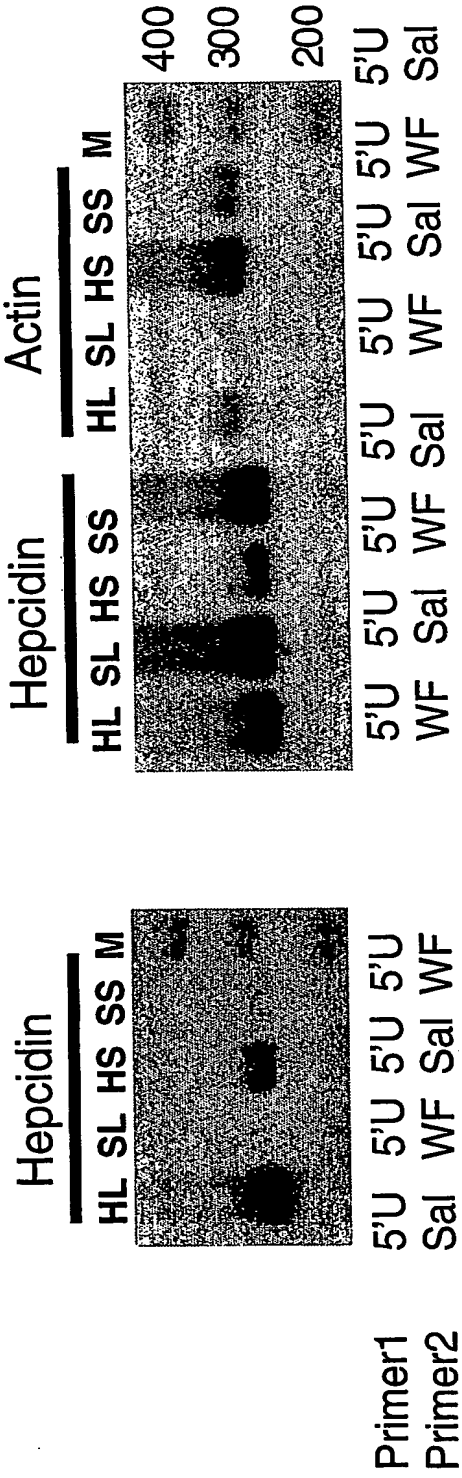
Hepcidin
HL SL HS SS M
5'U 5'U 5'U 5'U
Sal WF Sal WF
Primer1
Primer2
Actin
HL SL HS SS M
400
300
200
5'U 5'U 5'U 5'U
WF Sal WF Sal

Fig. 2

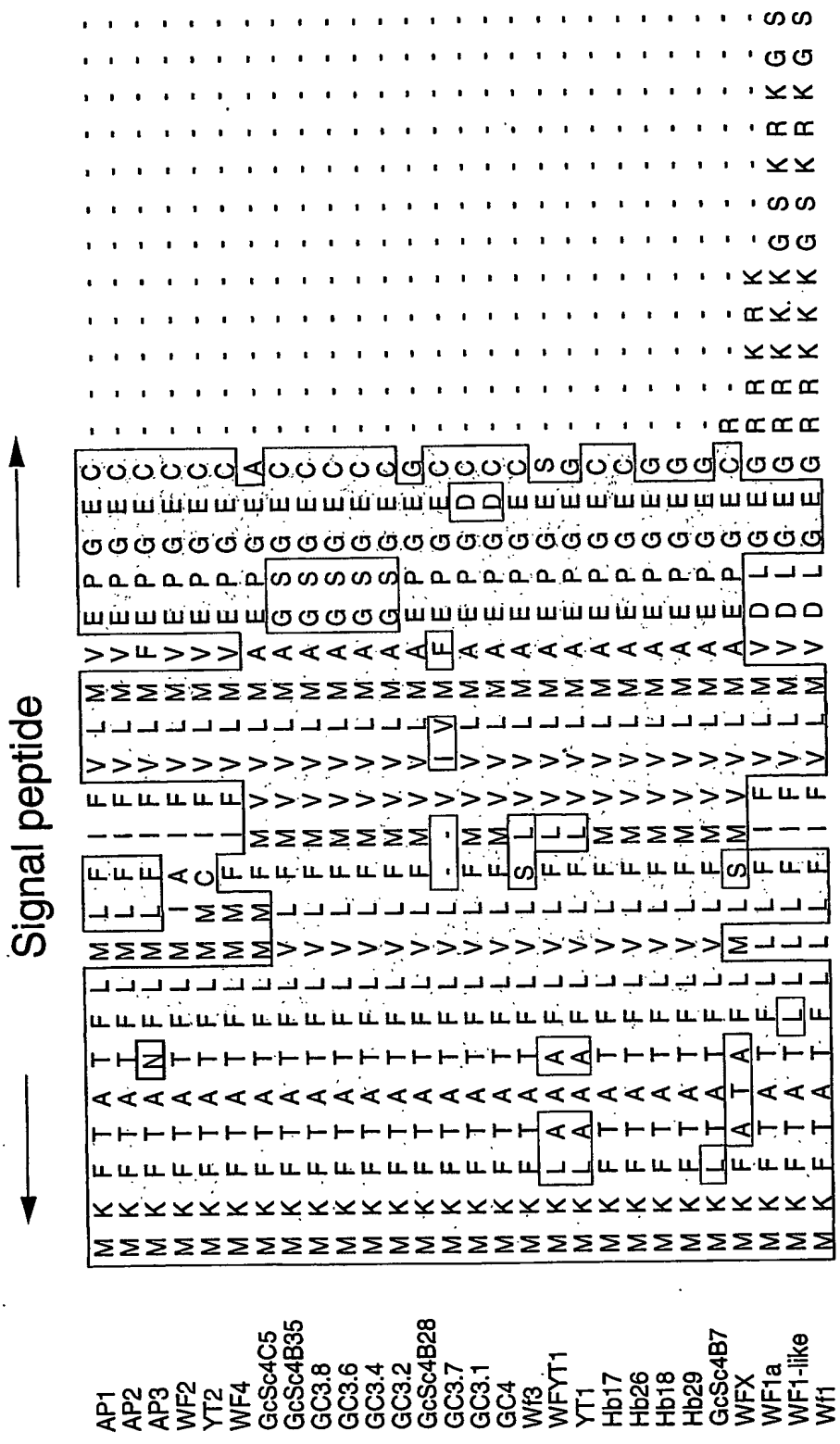
Signal peptide
AP1
AP2
AP3
WF2
YT2
WF4
GcSc4C5
GcSc4B35
GC3.8
GC3.6
GC3.4
GC3.2
GcSc4B28
GC3.7
GC3.1
GC4
Wf3
WFYT1
YT1
Hb17
Hb26
Hb18
Hb29
GcSc4B7
WFX
WF1a
WF1-like
Wf1

Fig. 3

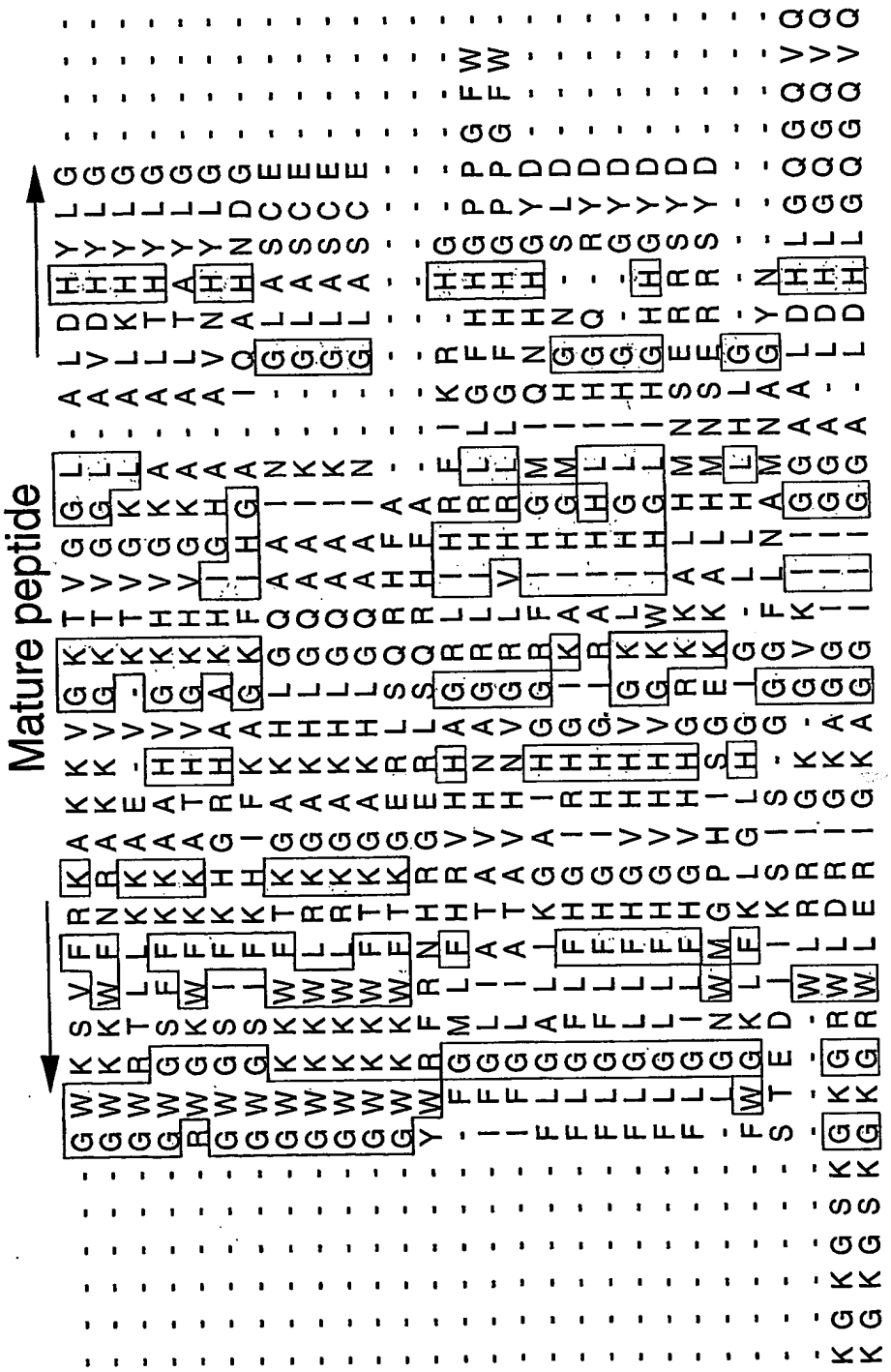

Fig. 3 (Cont.)

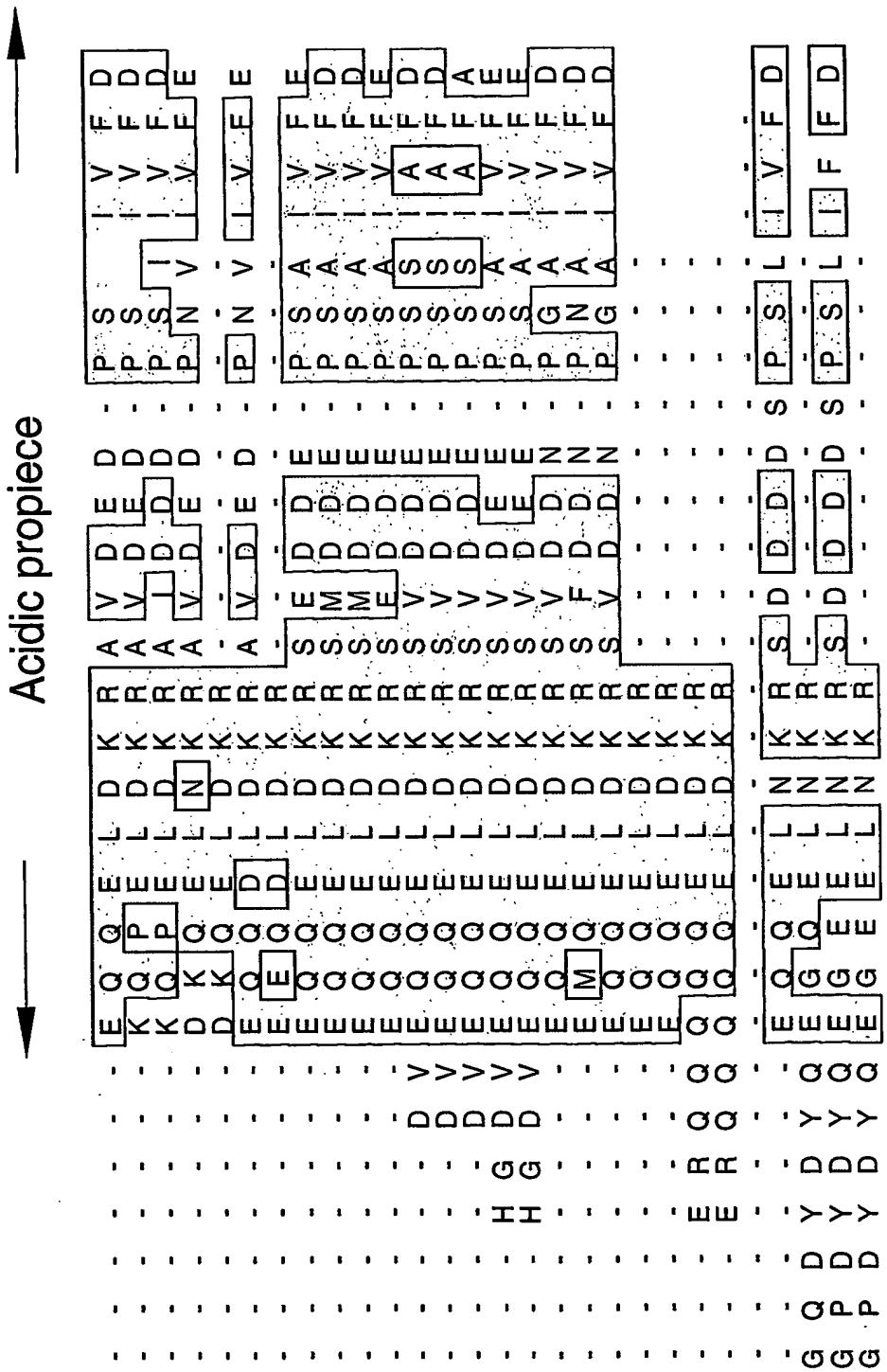

Fig. 3 (Further Cont.)

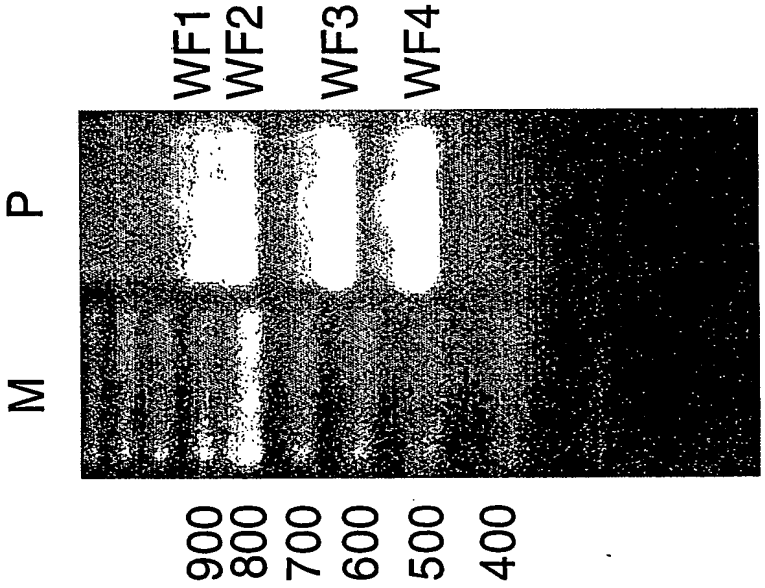

Fig. 4

```
                Primer PL1
1   GAATTCGCCCTTGCCCACTTTGTATTCGCAAG gtaatatcaatatttttca

101 ctgtataatgcaaatgttaatgatctttatttttctgttttttttttgtaga
1

189 TTC GTC CTC ATG GTT GAA CCT GGA GAG TGT GGT TGG GGA
13   F   V   L   M   V   E   P   G   E   C   G   W   G

267 ttaattagcttttaactttgcaaatattgtttttttttttaacagctggaaa

367 atctaaataacaacctaaaaggcctttgattagcatgtttcttcaataaaat

467 gtttgtttttacacag CT GCC AAG CAT ATT GGC CAT GCA GCC
35                      A   K   H   I   G   H   A   A

555 ttatcaccagtattgttattgacaacttctcttttttcctgctgatccgactc
45

643 GAC AAG CGC GCA GTC GAT GAA GAC CCA AAT GTT ATT GTT
54   D   K   R   A   V   D   E   D   P   N   V   I   V
```

Fig. 5

```
aattcatttagacgagaccaacctttttgggaaatctgctcagcttatta 100

 ATG AAG TTC ACT GCC ACC TTC CTC ATG ATG TTC ATC  188
  M   K   F   T   A   T   F   L   M   M   F   I   12

AGC ATT TTT AAG CAT GGT CGT CAT G gtaaagtcacggaa  266
 S   I   F   K   H   G   R   H   A                34

ctcacaaaaataaatagccgatatatttggccaattataatcactttg  366

gattgaacactacttaaaggtatgtataaaacatcatcatgtgttttt  466

GTT AA gtaaggacttctaccattattactgtataattttgatagta  554
 V   N                                            44

atccgcag T CAT TAC CTT GGC GAG CAG CAA GAT CTC     642
         H   Y   L   G   E   Q   Q   D   L         53

TTT GAA TGAagaaatcgccttgaaggagccttcagaagggcgaattc 728
 F   E   *            Primer PL2                   68
```

Fig. 5 (Cont.)

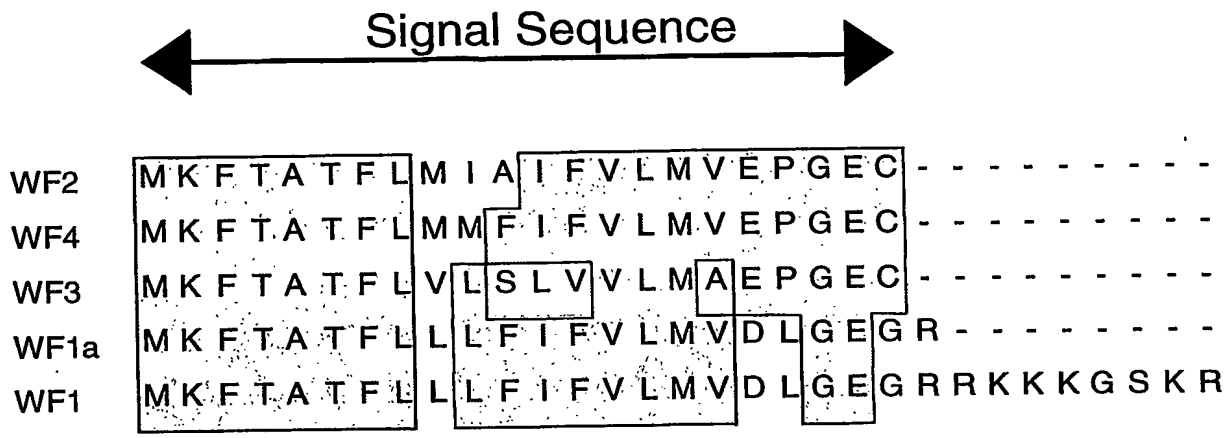

Fig. 6

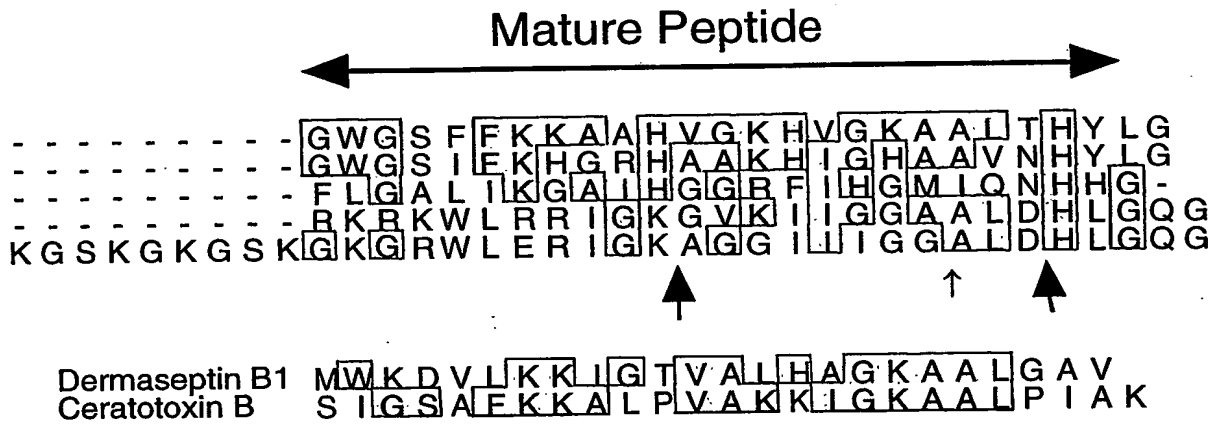

Fig. 6 (Cont.)

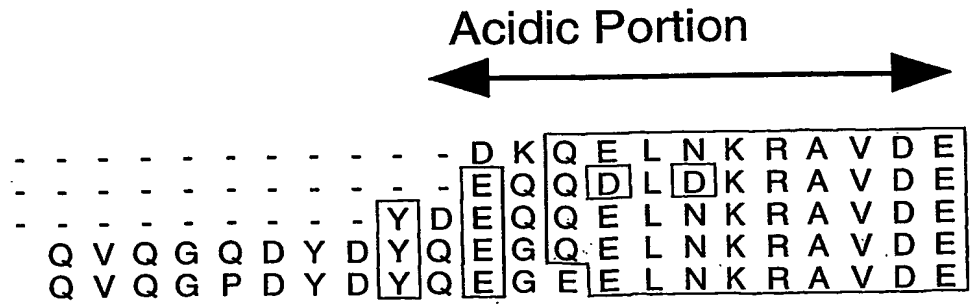

Fig. 6 (Cont.)

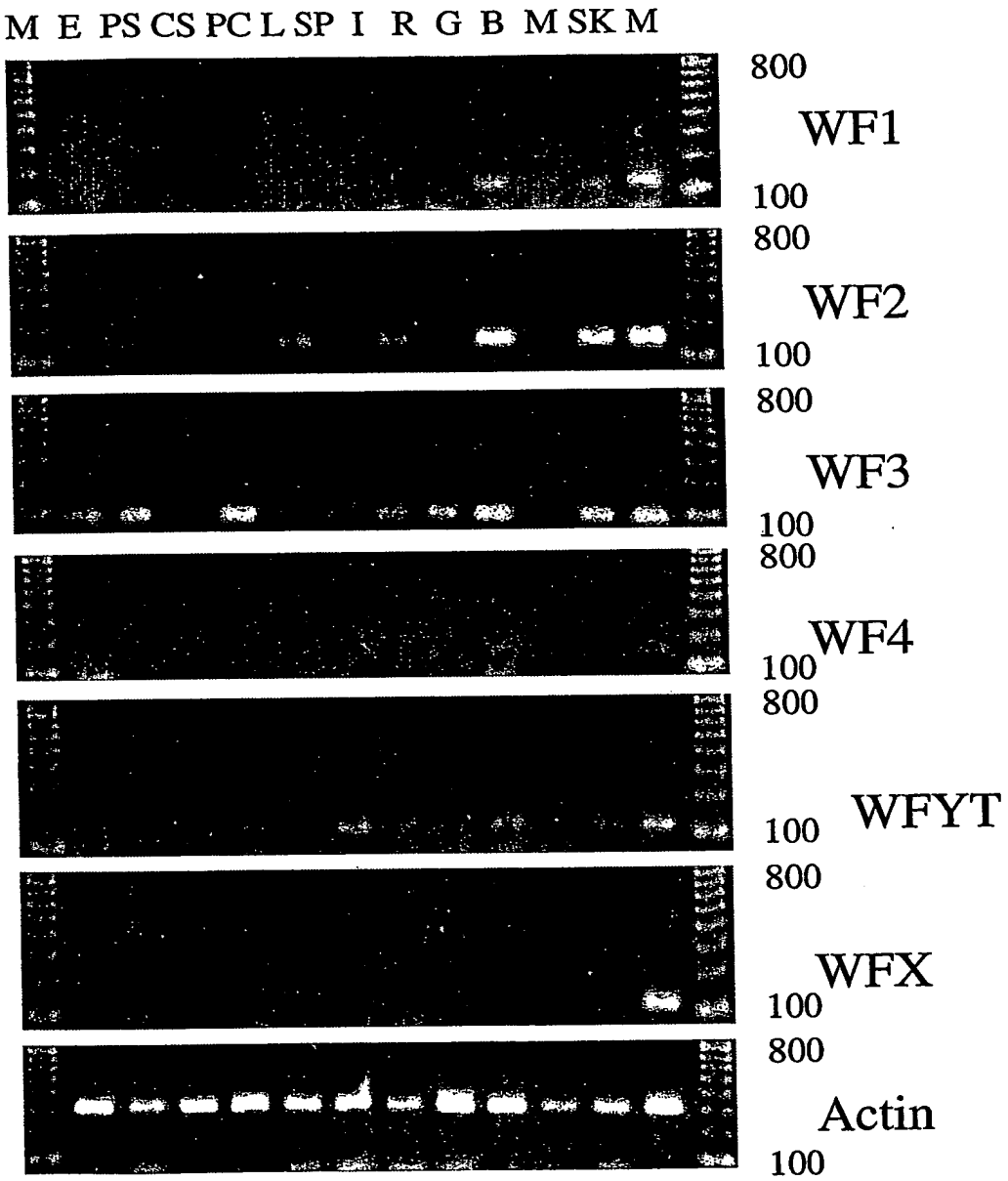
M E PS CS PC L SP I R G B M SK M
800
WF1
100
800
WF2
100
800
WF3
100
800
WF4
100
800
WFYT
100
800
WFX
100
800
Actin
100

Fig. 7

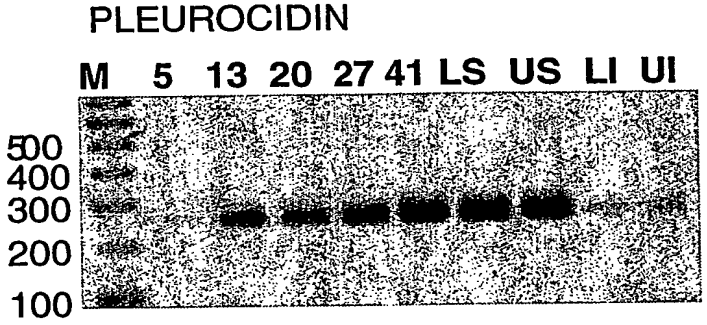
PLEUROCIDIN
M 5 13 20 27 41 LS US LI UI
500
400
300
200
100

Fig. 8a

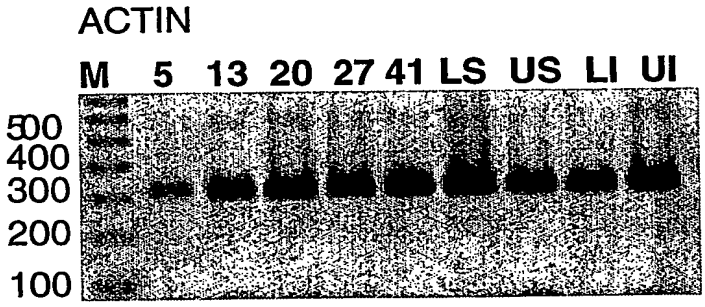
ACTIN
M 5 13 20 27 41 LS US LI UI
500
400
300
200
100

Fig. 8b

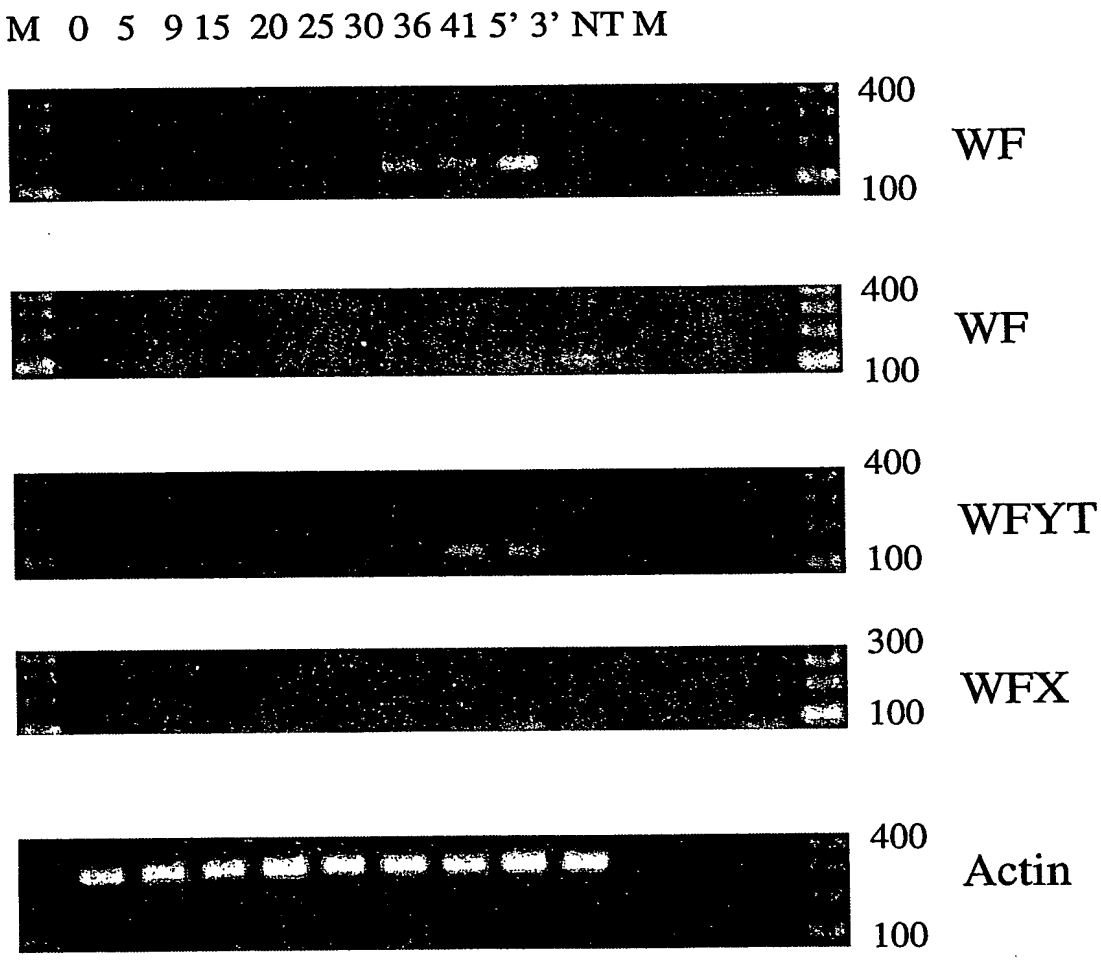
M 0 5 9 15 20 25 30 36 41 5' 3' NT M
400
100
WF
400
100
WF
400
100
WFYT
300
100
WFX
400
100
Actin

Fig. 9

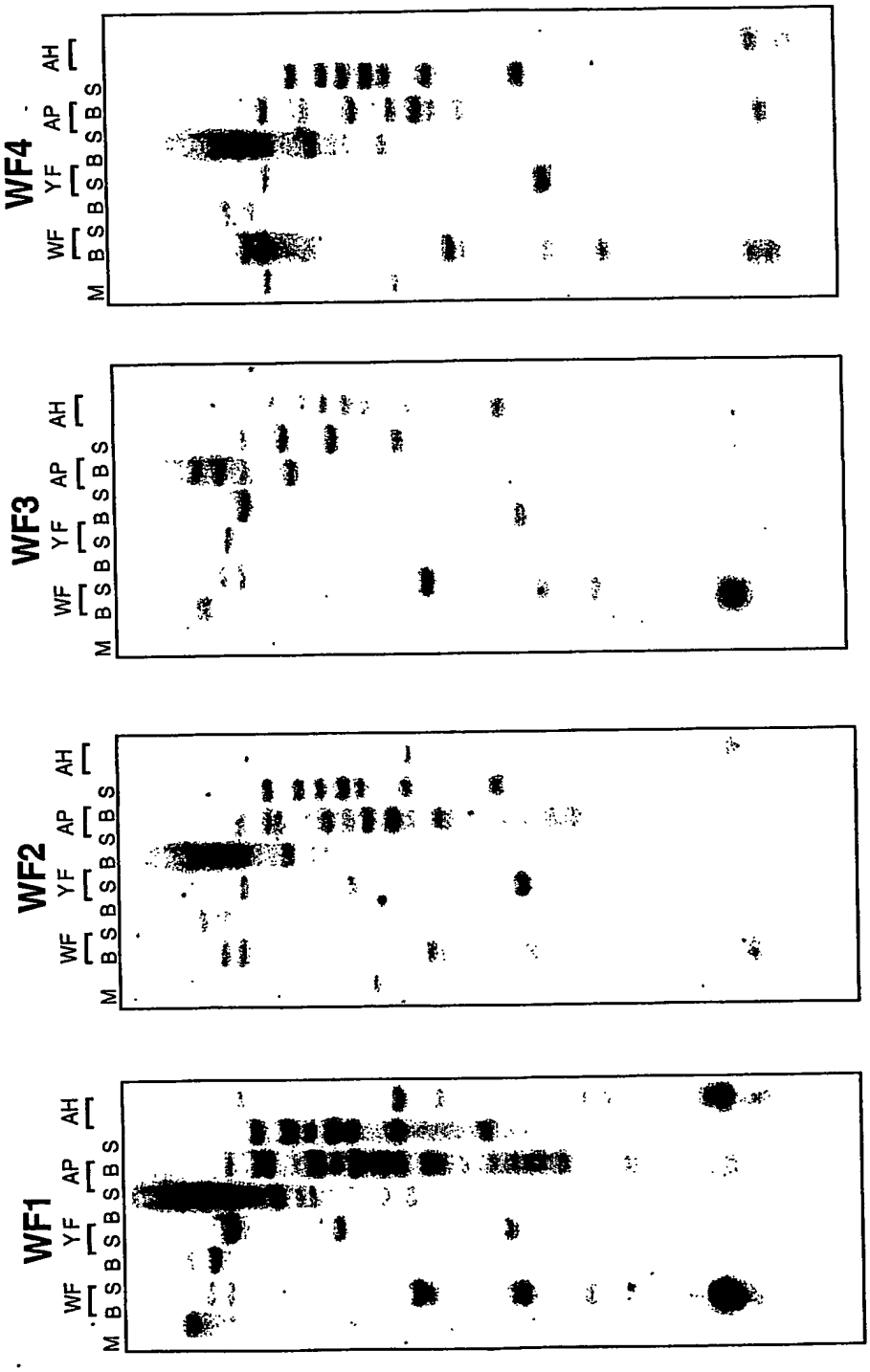

Fig. 10

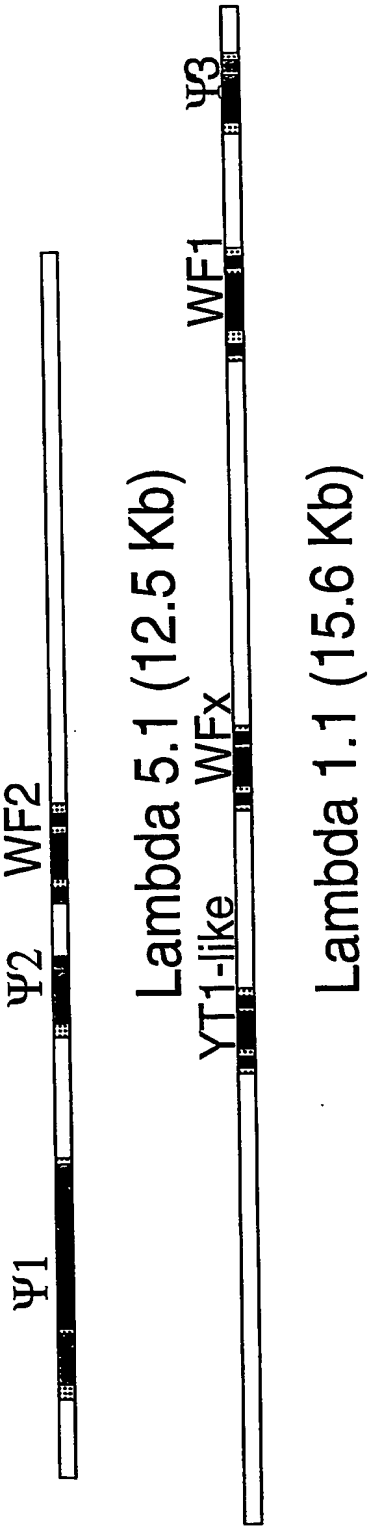

Fig. 11

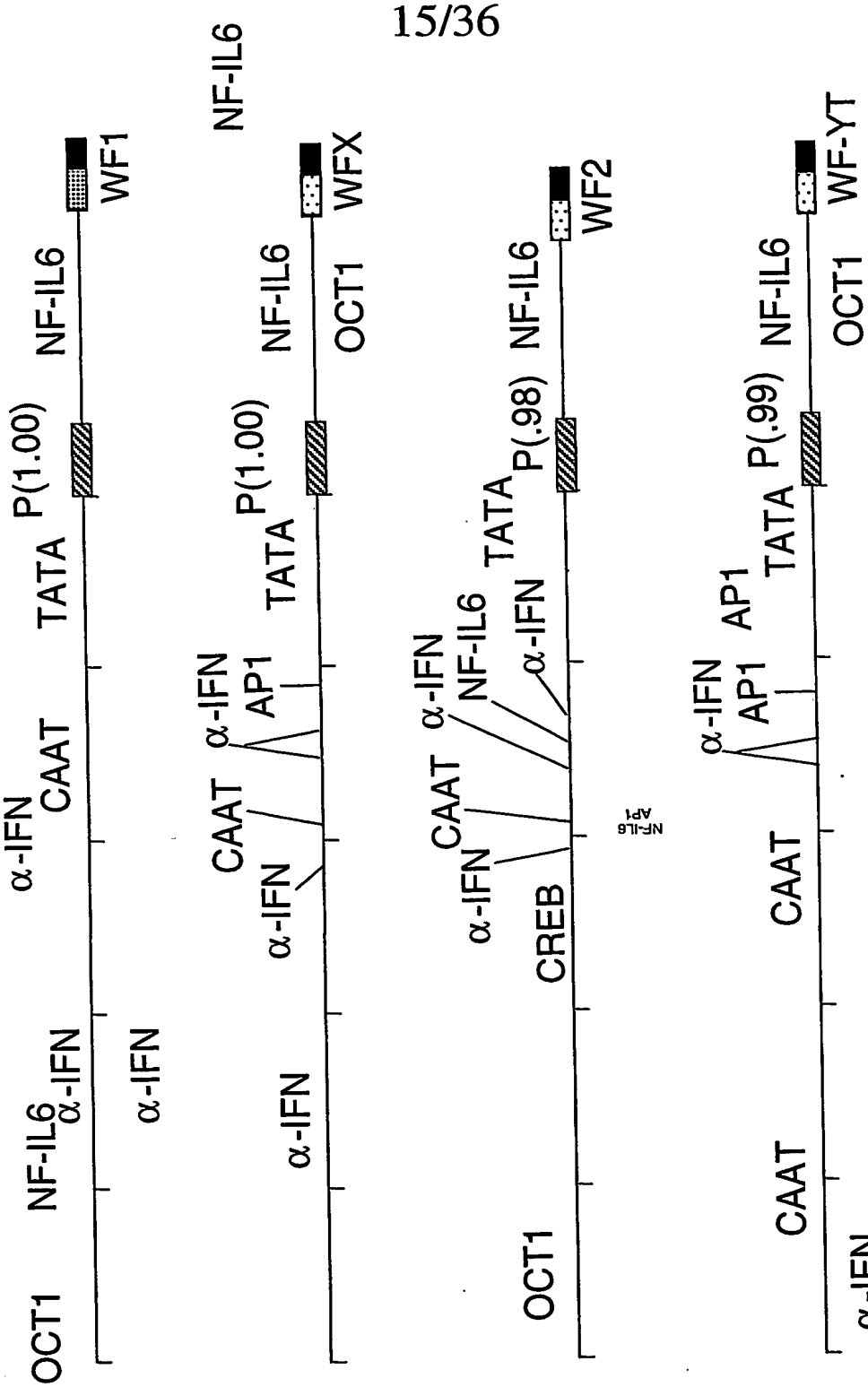
OCT1
NF-IL6
α-IFN
α-IFN
α-IFN
CAAT
TATA
P(1.00)
NF-IL6
WF1
OCT1
α-IFN
α-IFN
CAAT
α-IFN
AP1
TATA
P(1.00)
NF-IL6
OCT1
WFX
OCT1
α-IFN
CREB
α-IFN
CAAT
α-IFN
NF-IL6
TATA
P(.98)
NF-IL6
WF2
CAAT
α-IFN
AP1
TATA
P(.99)
NF-IL6
OCT1
WF-YT

Fig. 12

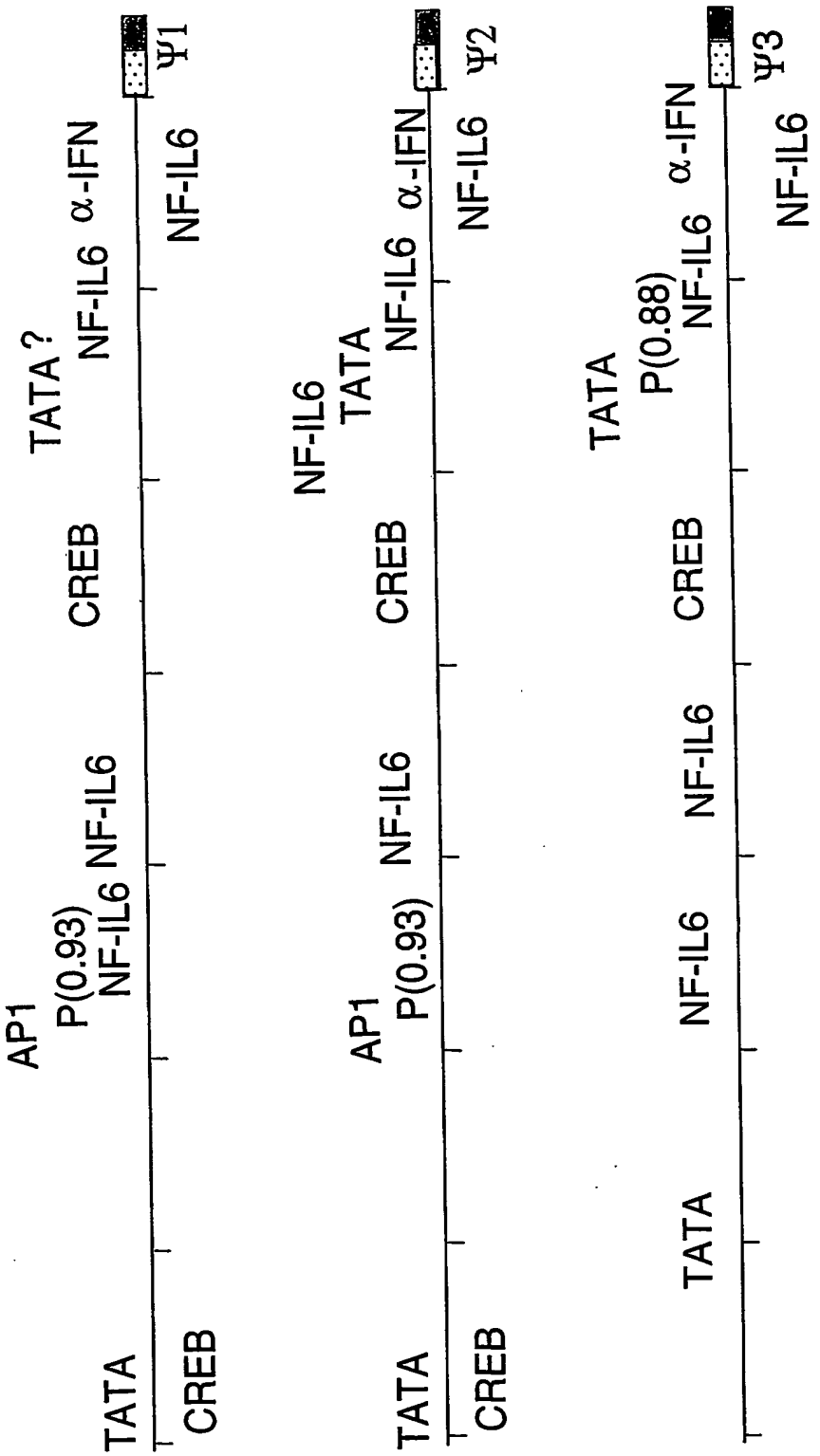
TATA
CREB
AP1
P(0.93)
NF-IL6
NF-IL6
CREB
TATA?
NF-IL6
α-IFN
NF-IL6
Ψ1
TATA
CREB
AP1
P(0.93)
NF-IL6
NF-IL6
CREB
NF-IL6
TATA
NF-IL6
α-IFN
NF-IL6
Ψ2
TATA
NF-IL6
NF-IL6
CREB
TATA
P(0.88)
NF-IL6
α-IFN
NF-IL6
Ψ3

Fig. 12 (Cont.)

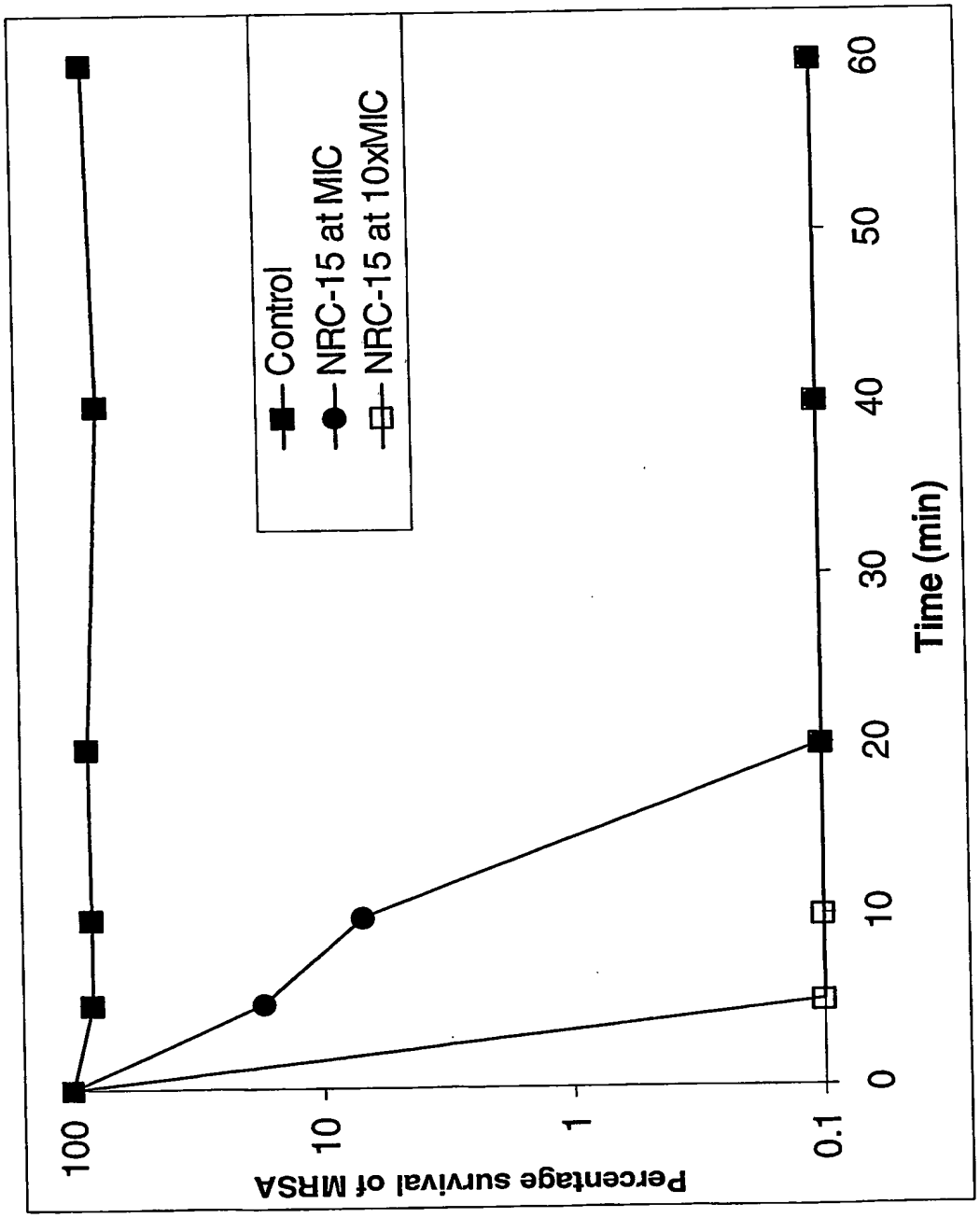
Control
NRC-15 at MIC
NRC-15 at 10xMIC
Percentage survival of MRSA
100
10
1
0.1
0
10
20
30
40
50
60
Time (min)

Fig. 13

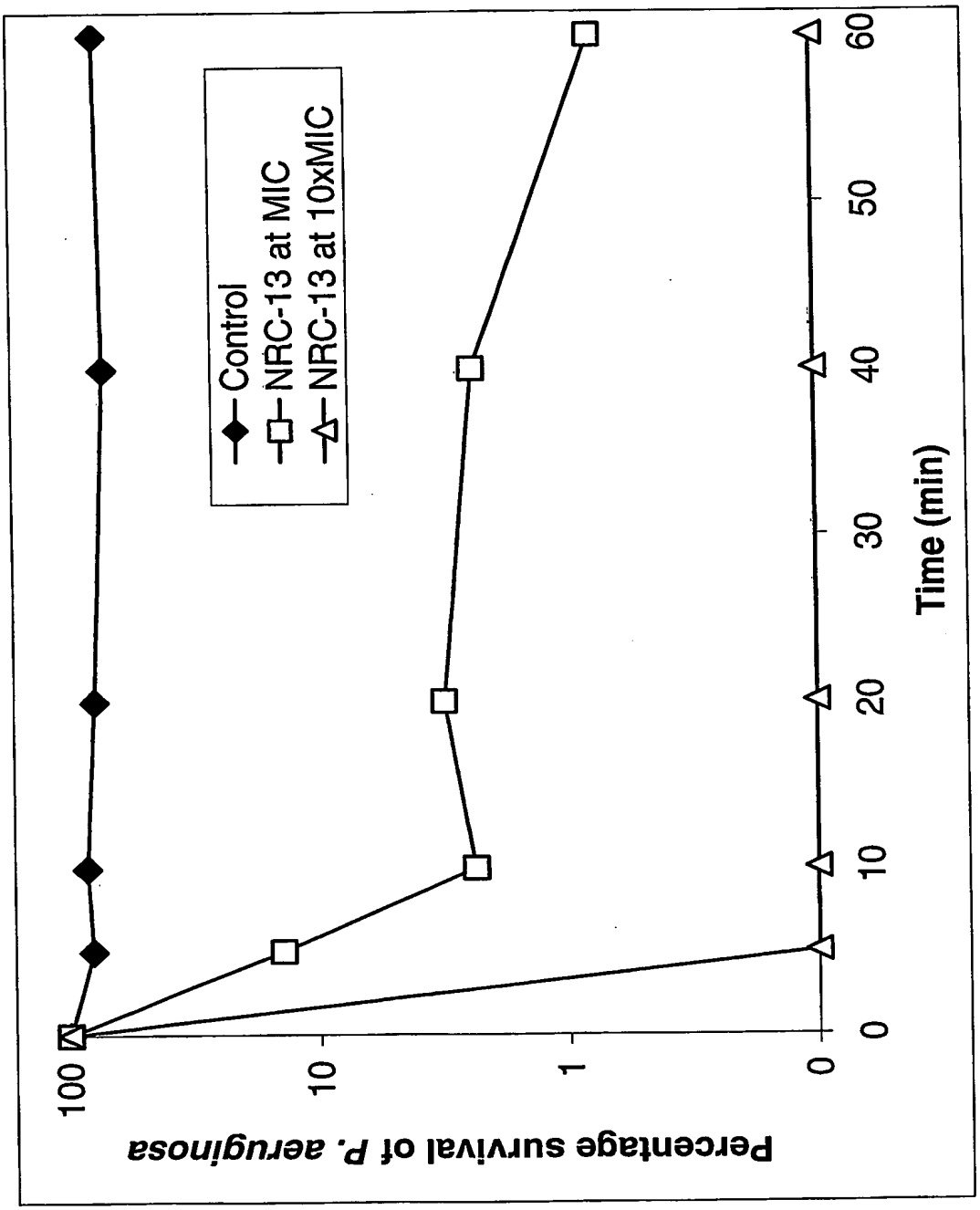
Percentage survival of P. aeruginosa
100
10
1
0
0
10
20
30
40
50
60
Time (min)
Control
NRC-13 at MIC
NRC-13 at 10xMIC

Fig. 14

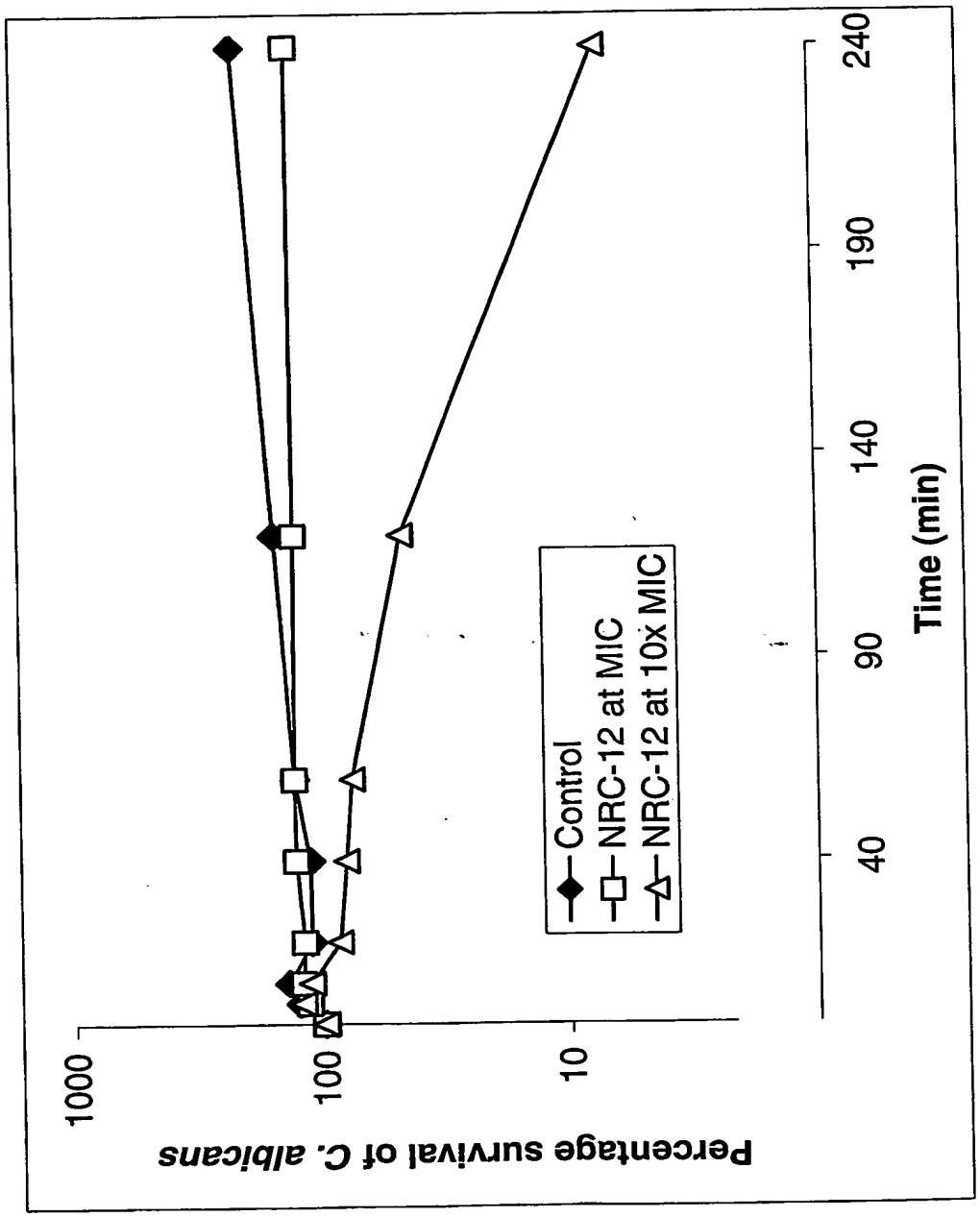
Percentage survival of C. albicans
1000
100
10
Time (min)
40
90
140
190
240
Control
NRC-12 at MIC
NRC-12 at 10x MIC

Fig. 15

```
  1 ctgacaccaaaagaacaatcaatcaactttggactcgtcttagtgcattg

101 atcttcctcataaactgtcacttcaatttcaactgatttcaacaggactt

195 TTT AGT GTT GCA GTG GTA CTC GTC ATT GCA TGT ATG TTC
    F   S   V   A   V   V   L   V   I   A   C   M   F

271 gtcaaattctccaacaccaacccactacaaacatgtgtgcatcgatttta

367 ACG GAG GAG GTT GGA AGC TTT GAC AGT CCA GTT GGG GAA
    T   E   E   V   G   S   F   D   S   P   V   G   E

442 gtacgttcaattgaatgaatgaattacgctaattacctttagcaaattaa

542 tagctgttaaccatttgattgtgagccgttagagggcttcagggcgagca

642 ctttttttcatattatttcttggcggggatacag GAG CCT TTC AGG
                                       E   P   F   R

724 TGC TGC AAC TGC TGT CAC AAC ATT GGC TGT GGC TTT TGC
    C   C   N   C   C   H   N   I   G   C   G   F   C

807 tgtttgcaatgttttctttctgagatgttgttttttgtgactatgataatga

907 ctttgaataacaaaaaaaaaaaaaaaa
```

Fig. 16a

```
aaaattgtgcgttggagagcgtcgcttttgggaacattgaagagttctg

ttaaataggctataaacttcctaaaaaaaacgaga ATG AAG GCC
                                       M   K   A

  ATC CTT GAA AGC ACC GCT GTT CCT TTC TCC GAG gtat
  I   L   E   S   T   A   V   P   F   S   E

gaggttggtcatgactcatttgtgcctaatgtctttgcag GTG CGA
                                          V   R

  CAT CAA CAG CCG GGC GGC GAG TCC ATG CAT CTG CCG
  H   Q   Q   P   G   G   E   S   M   H   L   P

cattttagtggttgcgttttaccctcggaatagaattagatcagtagcgc

gtgtgcaacgtggttgtgaagtggagatatacttacttgcttgttccctc

 TTC AAG CGT CAG ATC CAC CTC TCC CTG TGC GGT TTG
 F   K   R   Q   I   H   L   S   L   C   G   L

  TGC AAA TTC TAA ggacctgcccgcaacattttctagtttgtaca
  C   K   F   *

 tttataaaatcacttcttattgtgacactttaaaaaaataaacacatt
```

Fig. 16a (Cont.)

```
  1 cgcccttaag ATG AAG ACA TTC AGT GTT GCA GTT GCA GTG
  1            M   K   T   F   S   V   A   V   A   V

 77 ACC GCT GTT CCT TTC TCC GAG GTG CGA ACG GAG GAG
 23 T   A   V   P   F   S   E   V   R   T   E   E

152 CCG GGC GGC ACG TCC ATG AAT CTG CCGgtacgttcaattt
 48 P   G   G   T   S   M   N   L   P

243 gtttcacccttggaattgaattagcccactagcgctagttgttaacca

343 tgaagtggagacttggacaaaaatacttaccatgtgcttgttcccacc
 57

438 AAG CGT CAG AGC CAC CTC TCC CTG TGC CGT TGG TGC
 62 K   R   Q   S   H   L   S   L   C   R   W   C

513 AAA TTC TGA ggacctgccagcaaagggcgaattcgtttaaaacac
 87 K   F   *
```

Fig. 16b

```
  GTG GTC GTC CTC GCA TGT ATG TTC ATC CTT GAA AGC
   V   V   V   L   A   C   M   F   I   L   E   S

GTT GAA AGC ATT GAC AGT CCA GTT GGG GAA CAT CAA CAG
V   E   S   I   D   S   P   V   G   E   H   Q   Q

agtgaatgaattaagtaattacctttagcaaattaacatctaagtggttgc

tttgattgtgagccggtagagagggcttcagggcgagtagtgtgaatacttg

tttttcattttcttttcttggctgagatacag ATG CAT TTC AGGTTC
                                 M   H   F   R   F

TGC AAC TGC TGT CAC AAC AAG GGC TGT GGC TTC TGC TGC
C   N   C   C   H   N   K   G   C   G   F   C   C
```

Fig. 16b (Cont.)

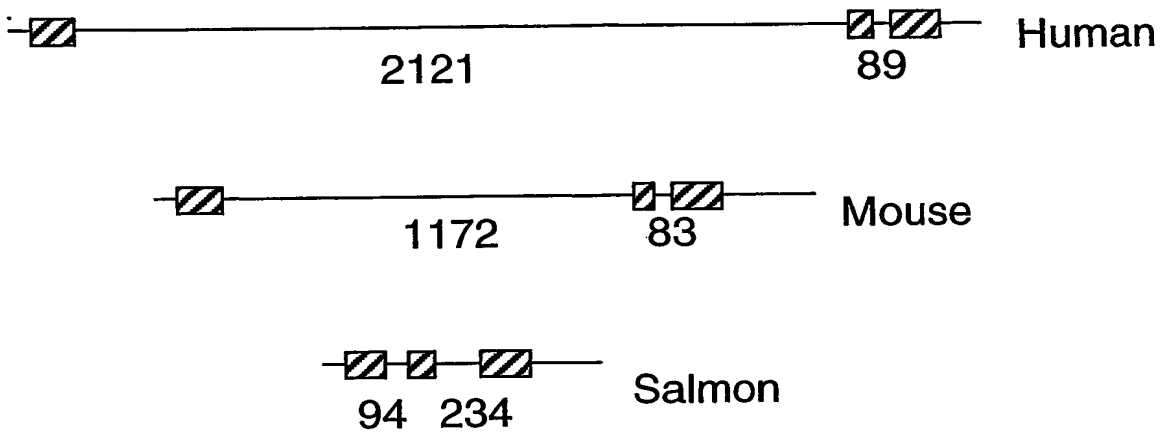

Fig. 16c

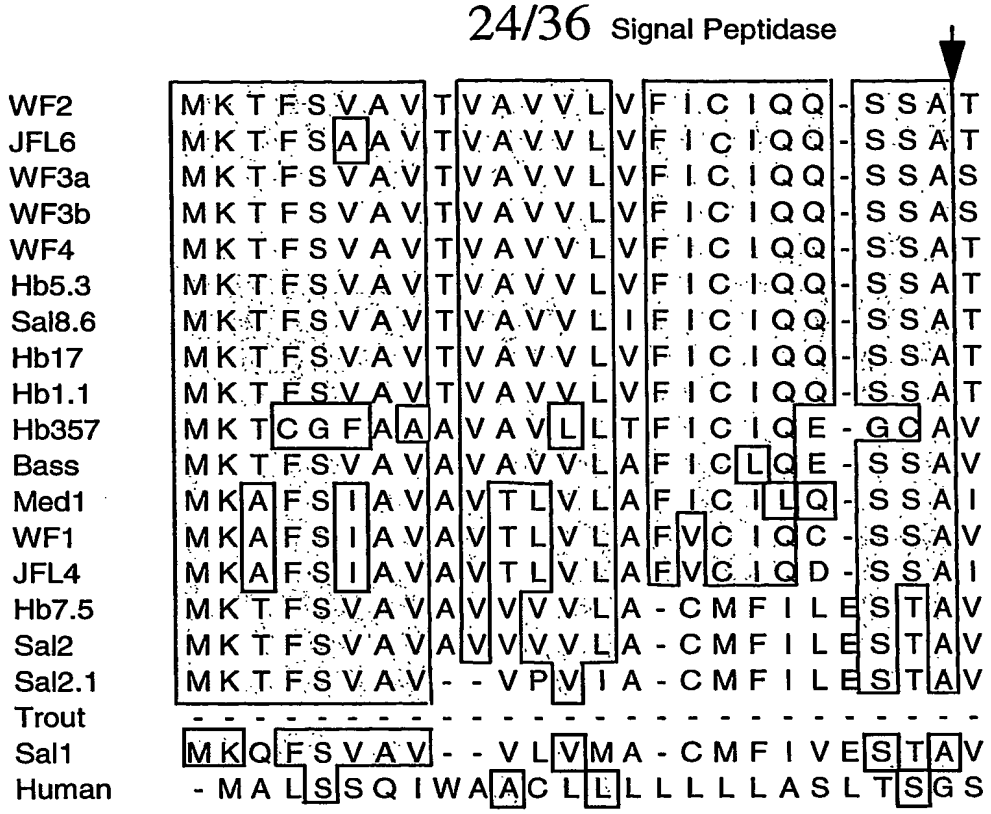

Fig. 17a

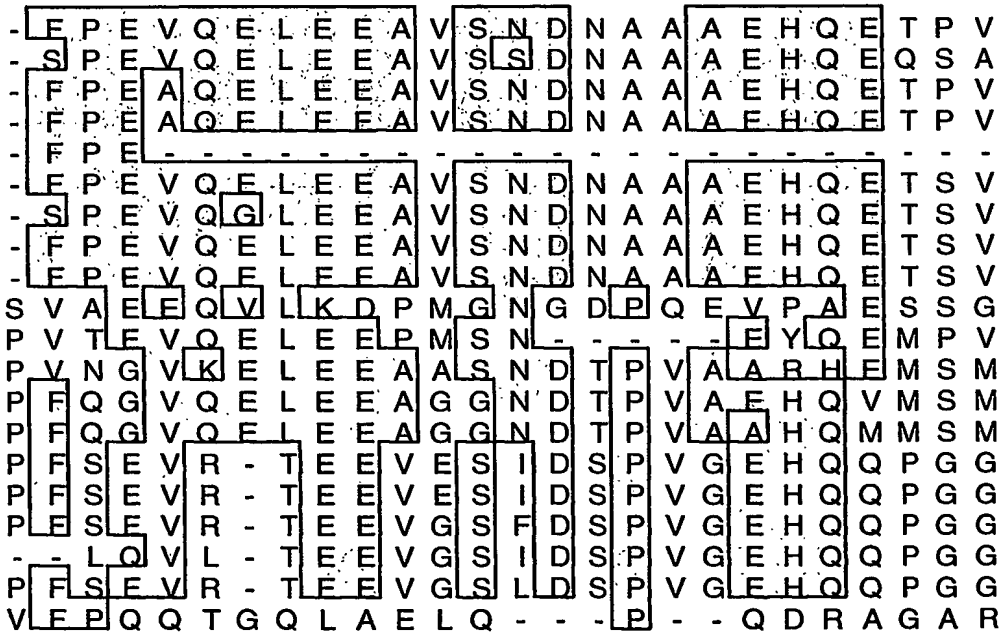

Fig. 17a (Cont.)

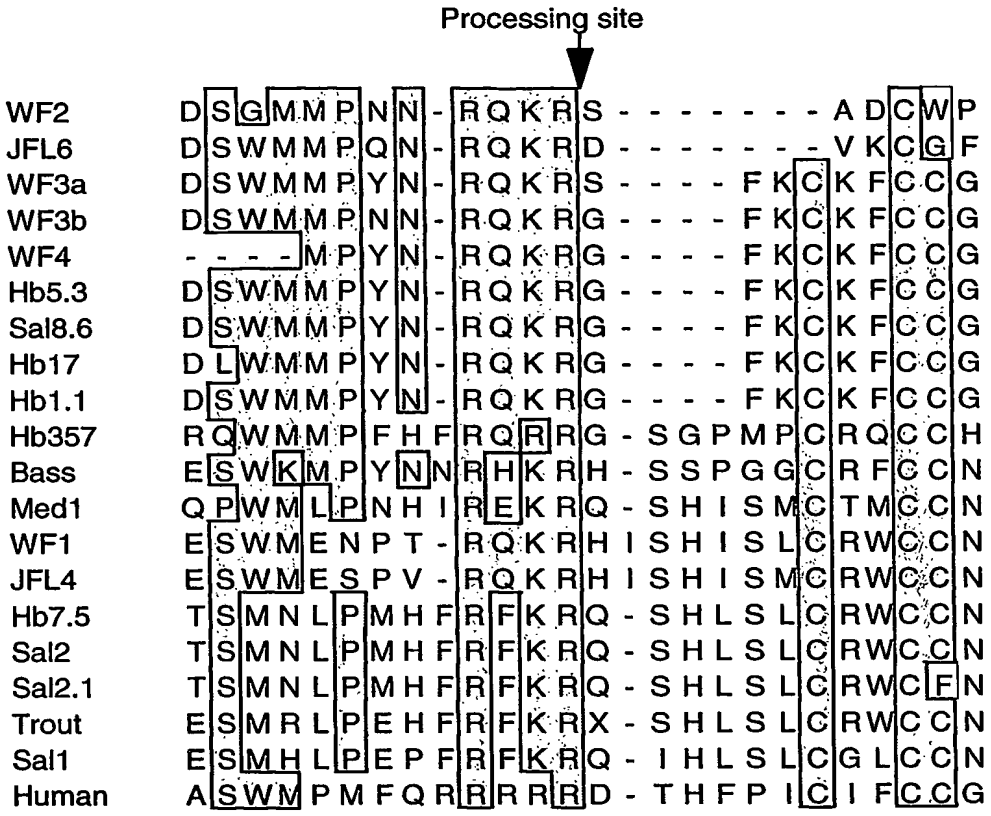

Fig. 17b

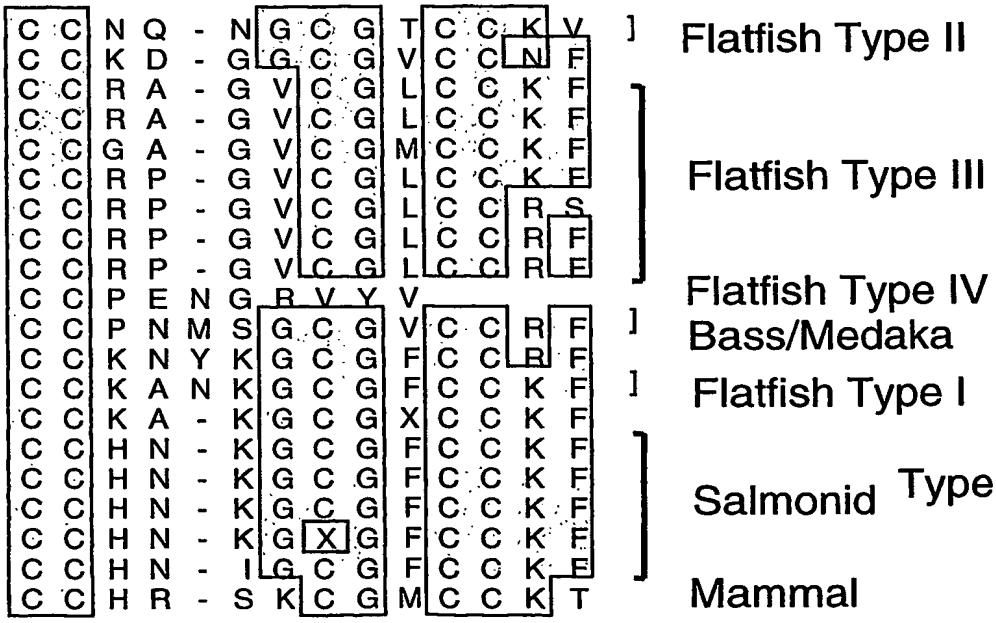

Fig. 17b (Cont.)

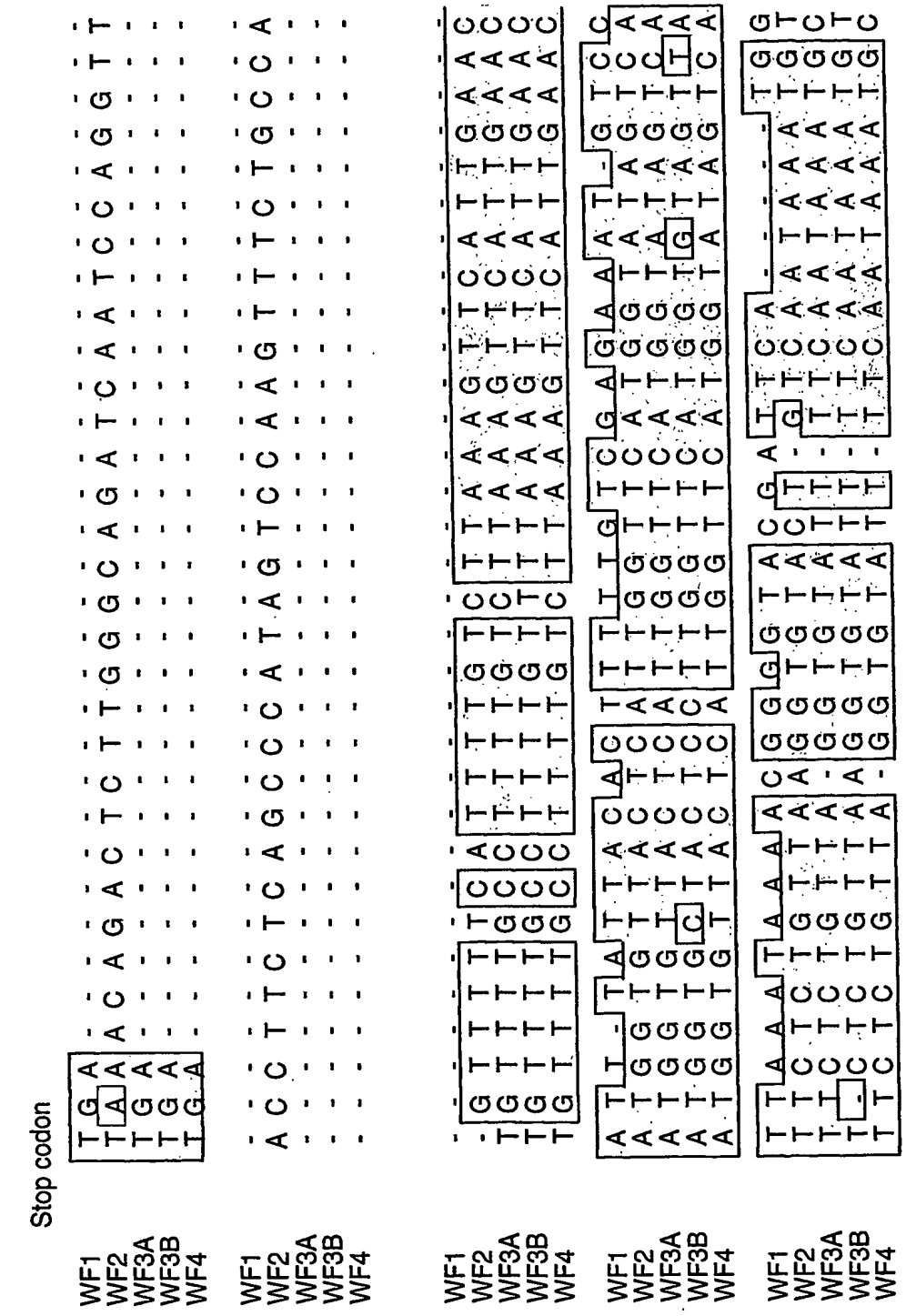

Fig. 18a

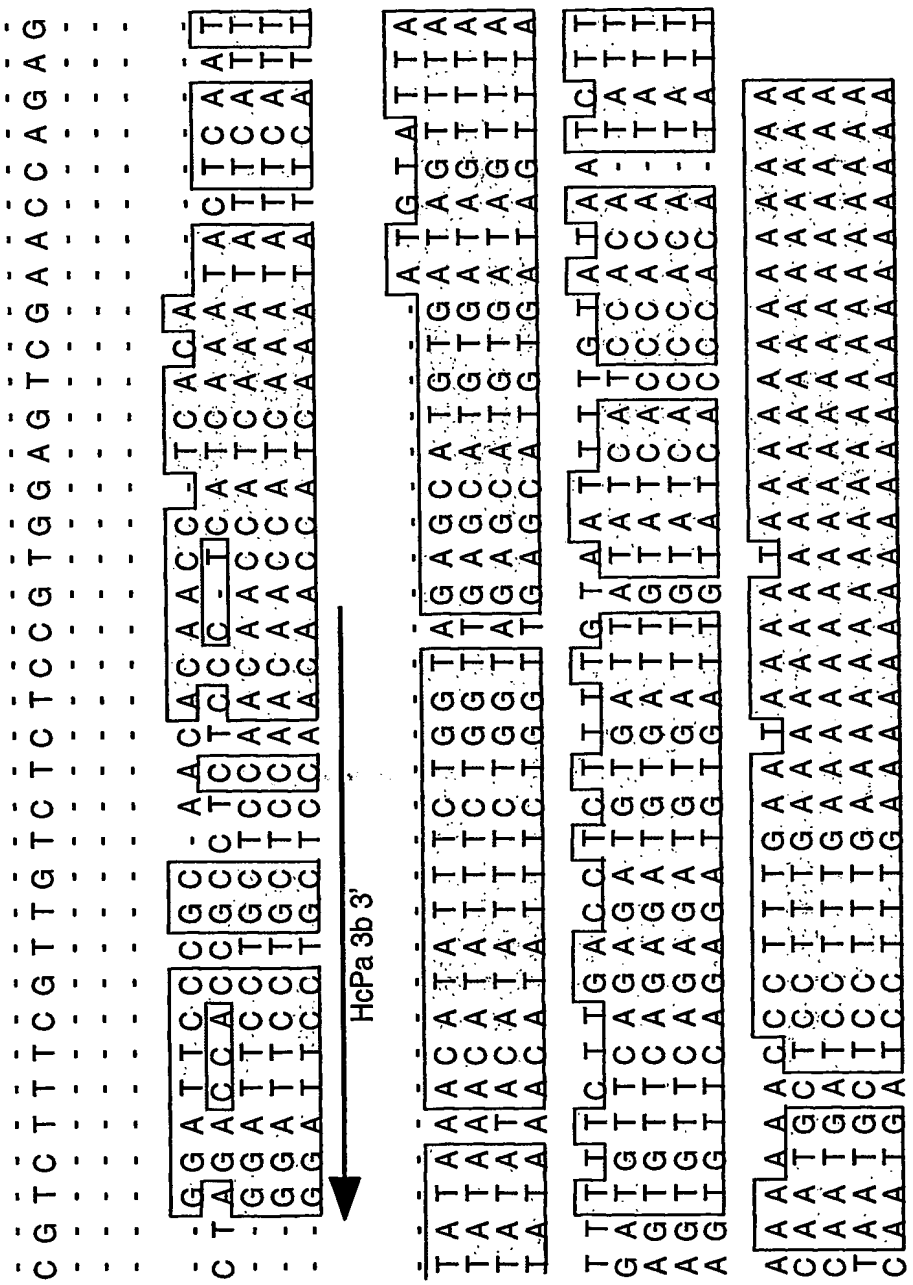

Fig. 18a (Cont.)

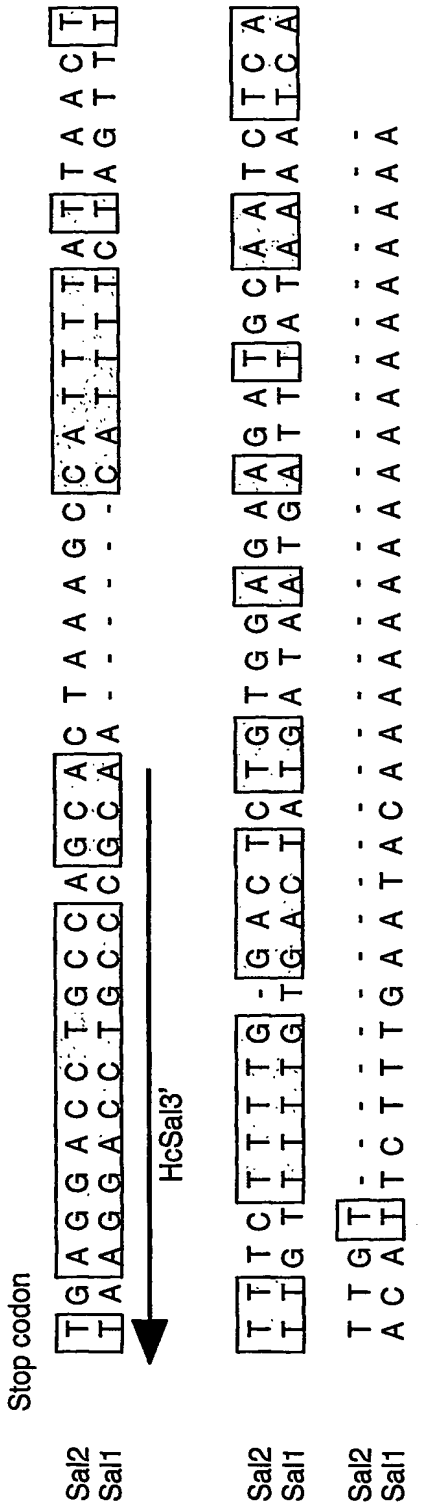

Fig. 18b

Fig. 18b (Cont.)

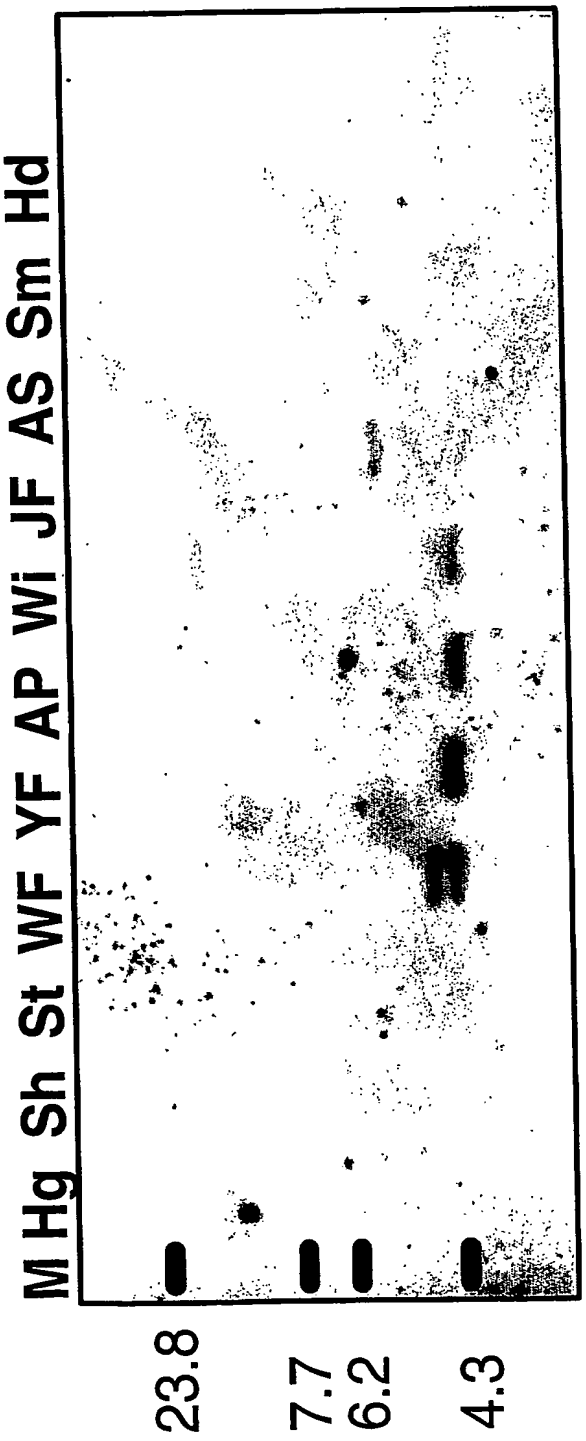

Fig. 19

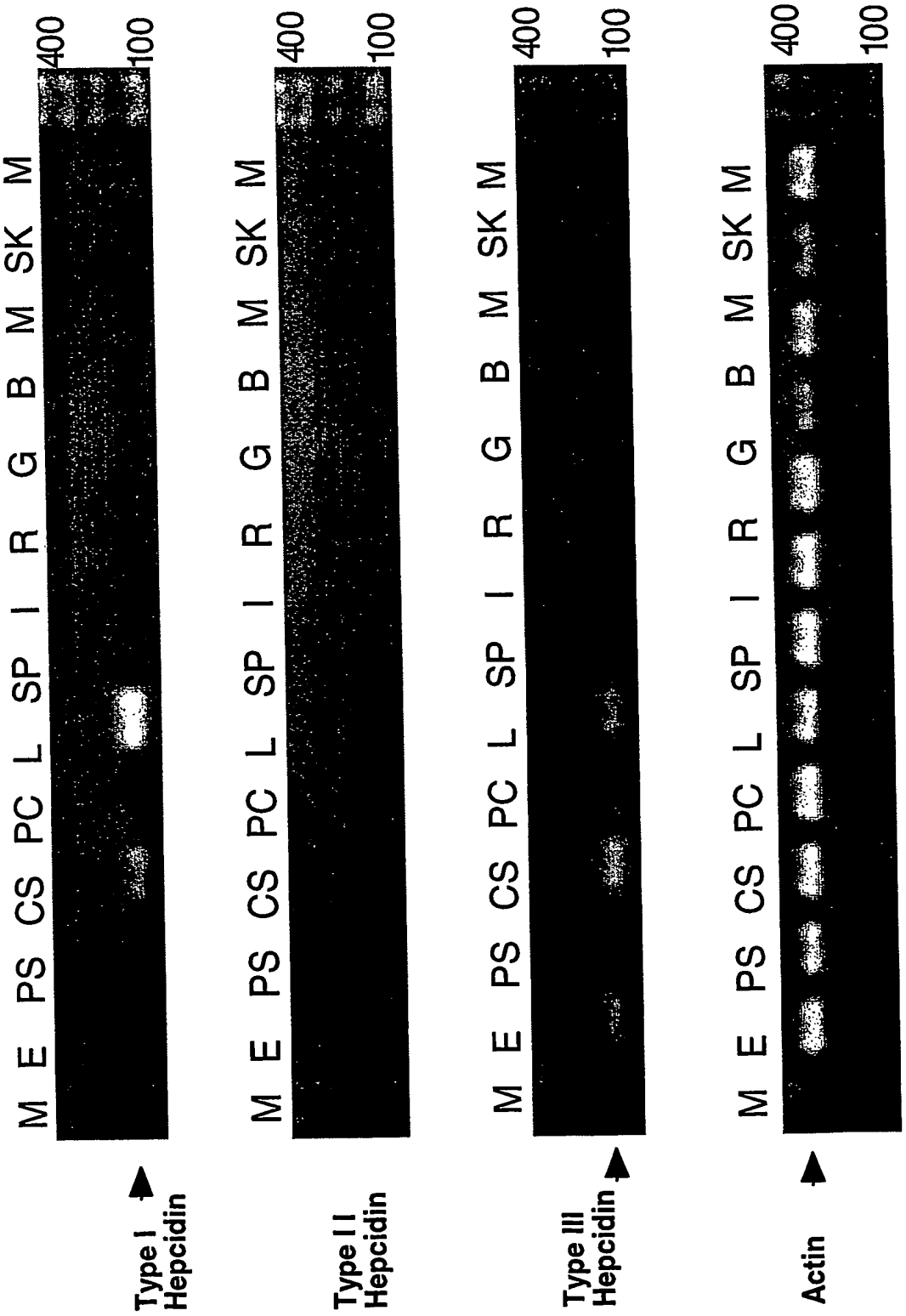
M E PS CS PC L SP I R G B M SK M
400
100
Type I Hepcidin
Type II Hepcidin
Type III Hepcidin
Actin

Fig. 20

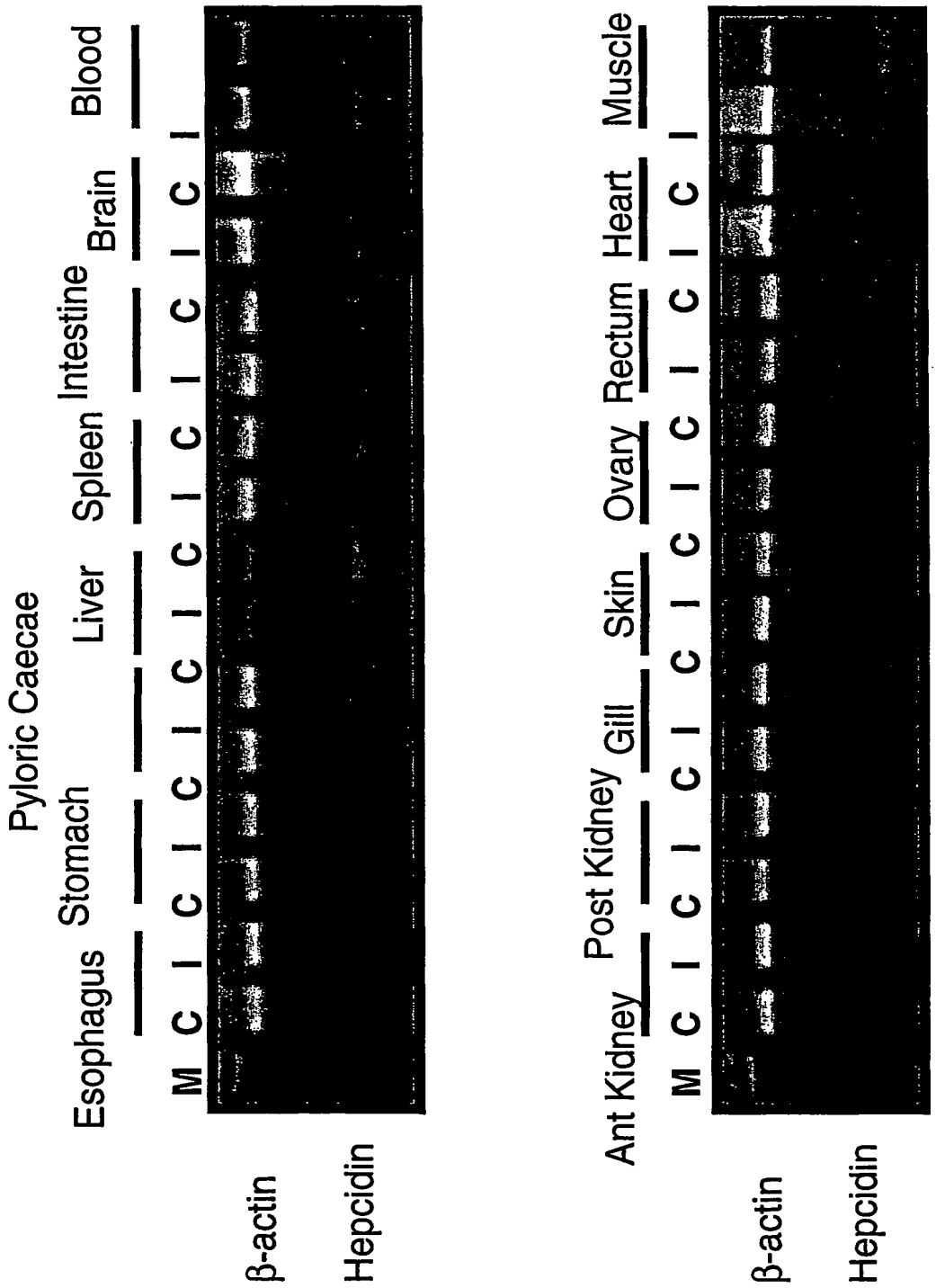
M C I C I C I C I C I C I C I
Esophagus
Stomach
Pyloric Caecae
Liver
Spleen
Intestine
Brain
Blood
β-actin
Hepcidin
M C I C I C I C I C I C I C I
Ant Kidney
Post Kidney
Gill
Skin
Ovary
Rectum
Heart
Muscle
β-actin
Hepcidin

Fig. 21a


Pyloric Caecae
Esophagus
Stomach
Liver
Spleen
Intestine
Brain
Blood
M C I C I C I C I C I C I
β-actin
Hepcidin
Ant Kidney
Post Kidney
Gill
Skin
Ovary
Rectum
Heart
Muscle
M C I C I C I C I C I C I
β-actin
Hepcidin

Fig. 21b

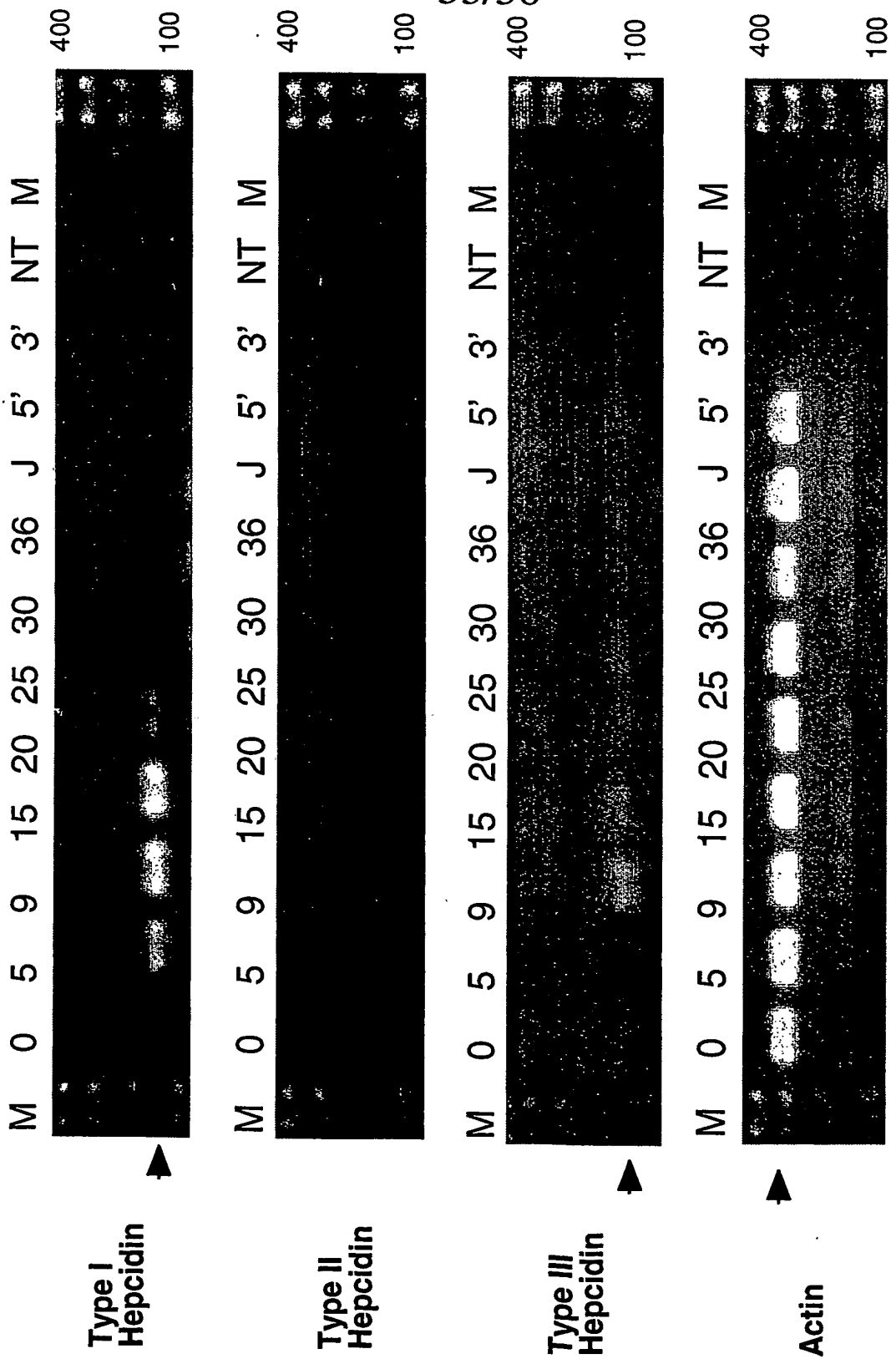
M 0 5 9 15 20 25 30 36 J 5' 3' NT M
400
100
Type I Hepcidin
Type II Hepcidin
Type III Hepcidin
Actin

Fig. 22

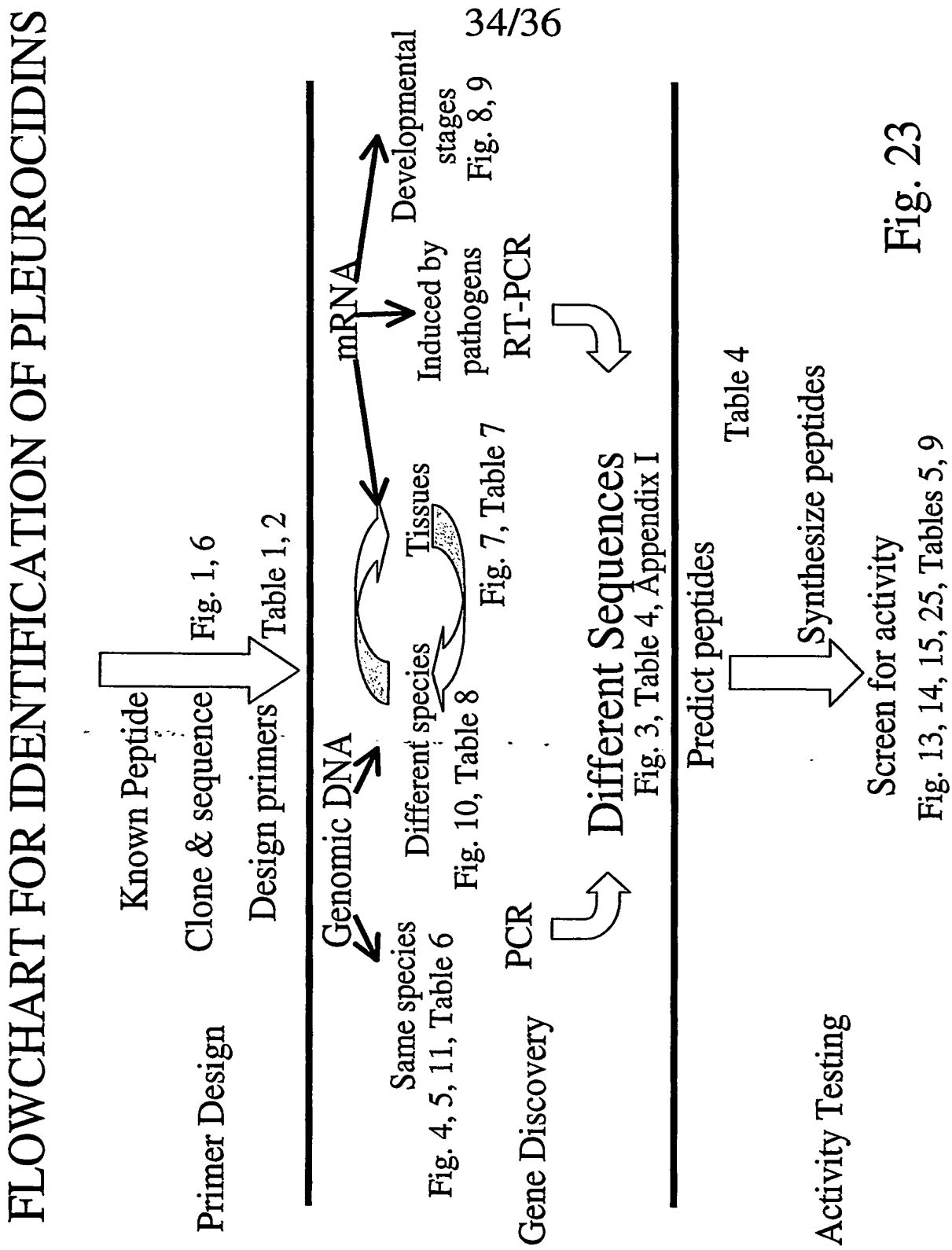
FLOWCHART FOR IDENTIFICATION OF PLEUROCIDINS
Primer Design
Known Peptide
Clone & sequence
Design primers
Fig. 1, 6
Table 1, 2
Gene Discovery
PCR
Genomic DNA
Same species
Fig. 4, 5, 11, Table 6
Different species
Fig. 10, Table 8
Tissues
Fig. 7, Table 7
mRNA
Induced by pathogens
RT-PCR
Developmental stages
Fig. 8, 9
Different Sequences
Fig. 3, Table 4, Appendix I
Predict peptides
Table 4
Activity Testing
Synthesize peptides
Screen for activity
Fig. 13, 14, 15, 25, Tables 5, 9

Fig. 23

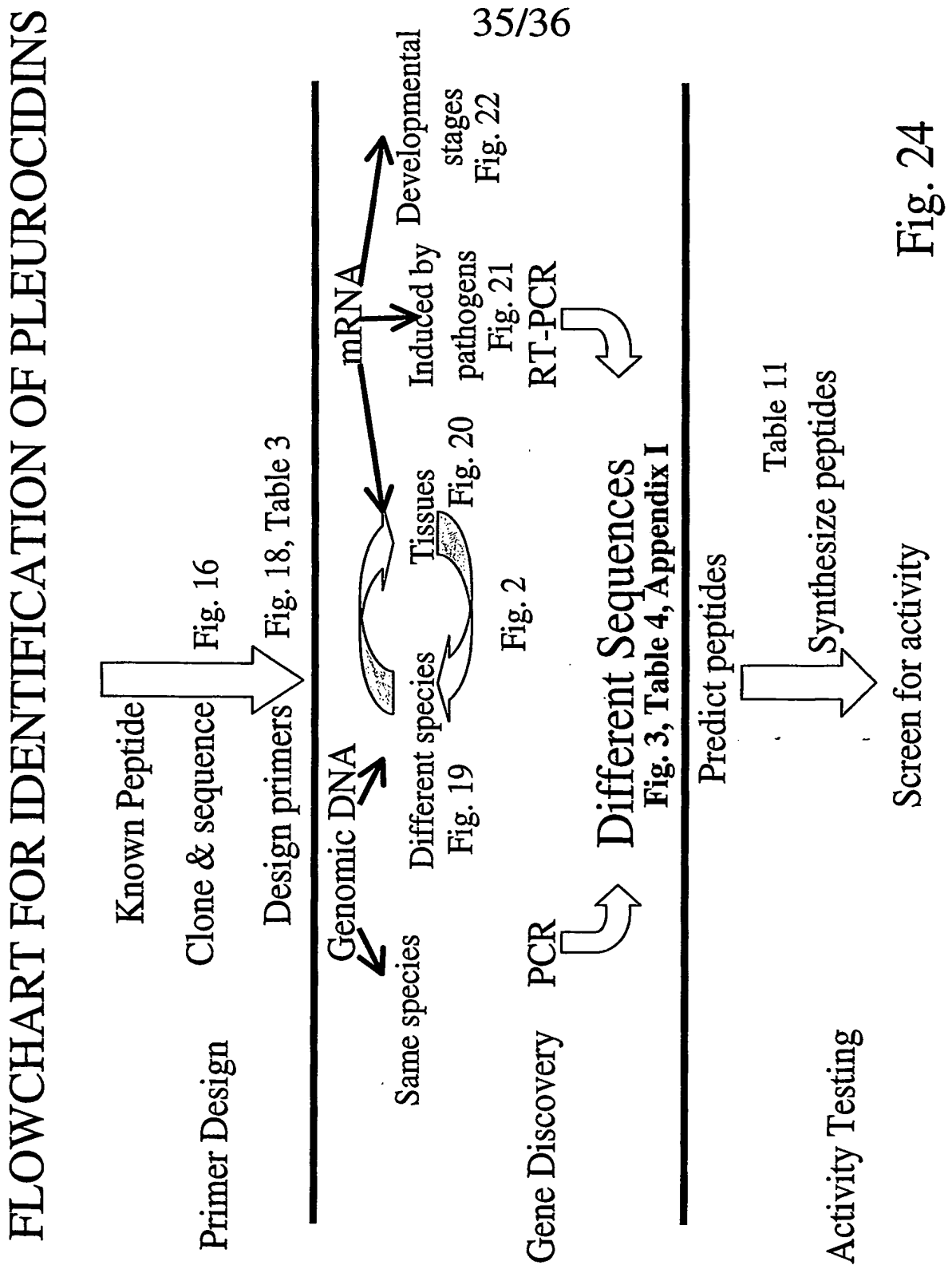
FLOWCHART FOR IDENTIFICATION OF PLEUROCIDINS
Known Peptide
Clone & sequence
Fig. 16
Primer Design
Design primers
Fig. 18, Table 3
Genomic DNA
Same species
Different species
Fig. 19
mRNA
Tissues
Fig. 20
Induced by pathogens
Fig. 21
Developmental stages
Fig. 22
Fig. 2
Gene Discovery
PCR
RT-PCR
Different Sequences
Fig. 3, Table 4, Appendix I
Predict peptides
Table 11
Synthesize peptides
Activity Testing
Screen for activity

Fig. 24

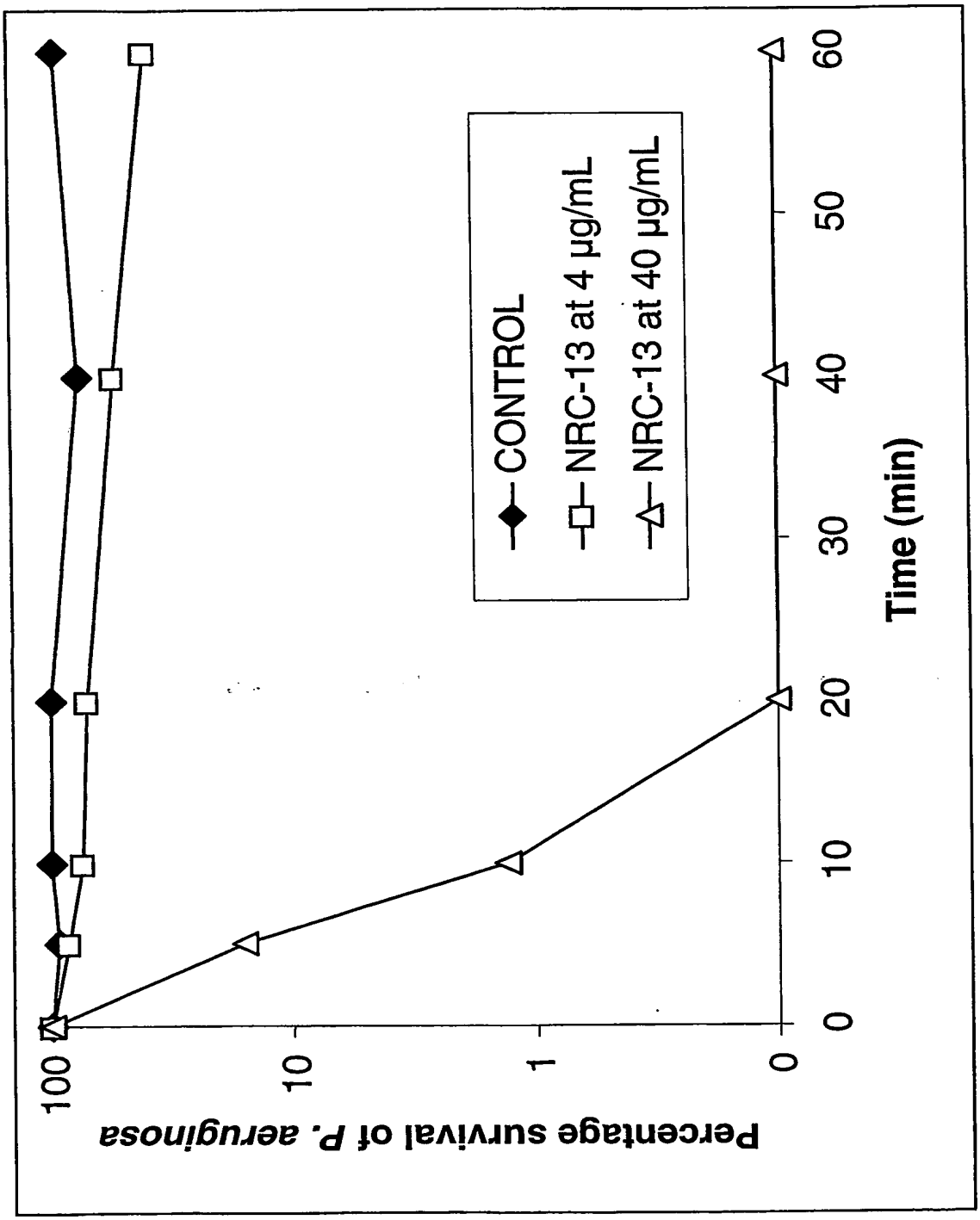
Percentage survival of P. aeruginosa
100
10
1
0
0
10
20
30
40
50
60
Time (min)
CONTROL
NRC-13 at 4 µg/mL
NRC-13 at 40 µg/mL

Fig. 25